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THE GENETIC NATURE OF DE VRIES'S MUTATIONS IN OENOTHERA LAMARCKIANA[1]

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To Professor Hugo de Vries, who has recently died after an unusually long and productive career, we owe the principal impetus to the experimental attack on the origin of new forms by mutation. His conceptions regarding the origin of new "elementary species" by mutation and the subsequent verification of many of his ideas, especially from the studies on Drosophila, are too well understood to need elaboration here. But while de Vries's studies on Oenothera led him very close to the currently accepted interpretation of mutation and showed that the process could be studied experimentally, the nature of the origin of the particular examples studied by him has been questioned practically from the start. It seemed probable that the new forms recurring in the progeny of *Oenothera Lamarckiana* result from some complex sort of segregation of genes, or aggregates of genes, already heterozygous in the parental form, and not from mutation in the strict sense. The inheritance of the variants themselves lent support to this interpretation, but only in the last few years have students of

[1] Based on a review presented to the Synapsis Club of the Citrus Experiment Station, Riverside, California, at a meeting commemorating the eighty-seventh birthday of Professor de Vries.

Oenothera genetics been able to give a satisfactory account of the origin of these derivative forms.

Practically all the different types of segregation that have been found to occur in the oenotheras are represented in the origins of the early "mutations" from *Oe. Lamarckiana*. Those obtained by de Vries in his original inbred line before 1901 are listed in Table 1, from which it can be noted that approximately 1.5 per cent. of the total progeny represent divergent types.

TABLE 1

Generation	*gigas*	*albida*	*oblonga*	*rubinervis*	*Lamarckiana*	*nanella*	*lata*	*scintillans*
I	..	..	..	..	9	..	..	..
II	..	..	..	..	15,000	5	5	..
III	..	..	..	1	10,000	3	3	..
IV	1	15	176	8	14,000	60	73	1
V	..	23	135	20	8,000	49	142	6
VI	..	11	29	3	1,800	9	5	1
VII	..	..	9	..	3,000	11	..	..
VIII	..	5	1	..	1,700	21	1	..

GENETIC CONSTITUTION OF *OE. LAMARCKIANA*

From the genetic studies of de Vries and others, but especially from the work of Renner, it became evident that many species of Oenothera regularly produce two types of gametes or complexes, as they are called by Renner. In many species, such as *Oe. muricata,* one complex functions only in the eggs and the other chiefly in the pollen. In other species, including *Oe. Lamarckiana,* both complexes are represented in about equal proportions in the functioning pollen and eggs. Outcrosses of *Oe. Lamarckiana* to other species consequently produce two distinct hybrid types, called twin hybrids, in the first generation, regardless of the direction in which the cross has been made. One twin receives the *gaudens* complex of *Oe. Lamarckiana,* the other the *velans* complex. Renner found that the two *Lamarckiana* complexes, *velans* and *gaudens,* differ from each other by many genes and

further that, as a rule, there is no mixing of genes between the complexes.

The reason for the lack of independent segregation of the genes belonging to the different complexes was not understood until Cleland began his cytological studies on the different oenotheras. In each species producing two types of gametes, he observed that the chromosomes fail to pair in the ordinary manner in the first meiotic division. Instead, the chromosomes are arranged end-to-end in large rings. The number of chromosomes present in such rings varies from species to species but is constant for any particular species or hybrid. *Oenothera Lamarckiana* was found to have a ring of twelve chromosomes and one chromosome pair as illustrated in Fig. 1a.

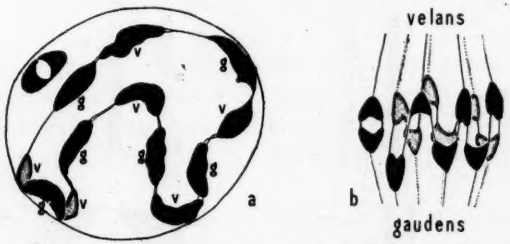

FIG. 1. Chromosome configurations at "diakinesis" (a) and early anaphase (b) of the first meiotic division in *Oe. Lamarckiana* (after Cleland —b somewhat diagrammatic). If the chromosomes (g) of the *gaudens* complex occupy alternating positions in the ring with those (v) of the *velans* complex, then all members of each complex will segregate as a unit in anaphase, as indicated in b.

Cleland observed further that when the ring chromosomes separate in anaphase of the first division those chromosomes lying immediately adjacent to one another regularly pass to opposite poles (Fig. 1b). If the chromosomes of the *gaudens* complex occupy alternate positions in the ring with the *velans* chromosomes lying between them, as indicated in the figure, it is evident that the regular zigzag disjunction of chromosomes will effectively separate the chromosomes of the two complexes. In this separation, the six *gaudens* chromosomes of the

ring all pass to one pole, and the six *velans* chromosomes to the other.

Why the chromosomes of a particular complex should occupy alternate positions in the ring, however, was not understood until Belling found similar ring-forming chromosomes in Datura. Belling's interpretation of the origin of these quadrivalent rings was that a reciprocal translocation had taken place between two non-homologous chromosomes in such a way that approximately one half of one chromosome became associated with one half of another non-homologous chromosome reconstituting a new chromosome, while the two remaining halves became associated to form a second reconstituted chromosome (Fig. 2a and 2b). If these two translocated chro-

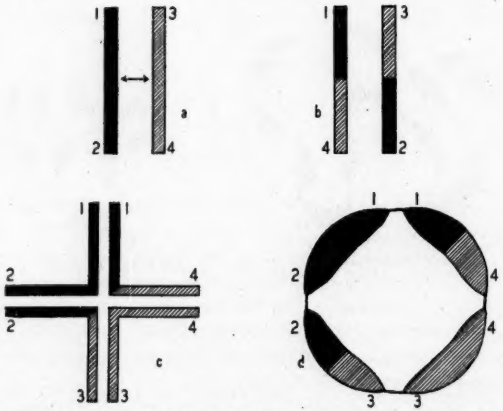

FIG. 2. The origin of ring-forming chromosomes as a result of reciprocal translocation. Two non-homologous chromosomes (a) are broken at the points indicated by the arrow. The "half" chromosomes are transposed and become reunited in two reconstituted chromosomes (b). In a hybrid receiving two such reciprocally translocated chromosomes (b) from one parent and two normal chromosomes (a) from the other, the pairing of homologous regions in the prophase of meiosis results in a cross-shaped figure composed of all four chromosomes (c) which will open out in diakinesis to form a ring of four chromosomes (d). In this and subsequent diagrams the equational split in the chromosomes is omitted for the sake of simplicity.

mosomes are present in one gamete and meet a gamete carrying the normal chromosomes, the synaptic pairing

between homologous regions of the chromosomes brings all four chromosomes together in a single cross-shaped figure, which, as the chromosomes pass into the diakinesis configuration, will form a ring of four chromosomes (Fig. 2c and 2d).

If this process of translocation were carried further, there should result chromosome rings with larger and larger numbers of members, such as have been found in the oenotheras. For example, if the chromosome labeled 1 · 2 in Figure 2a underwent reciprocal translocation with a third chromosome, say 5 · 6, to give two new chromosomes, 1 · 5 and 2 · 6, then a hybrid between a race carrying this translocation and one carrying the translocated chromosomes of Fig. 2b would have a ring of six chromosomes. The chromosomes in this ring of six would be 1 · 4, 4 · 3, 3 · 2, 2 · 6, 6 · 5, 5 · 1. Then, if a further translocation took place between one of the chromosomes of this ring of six and some chromosome not in the ring, a ring of eight chromosomes should result. This process might go on until all the chromosomes present were in a single ring.

It can now be shown that the large chromosome rings in the oenotheras have actually arisen from such a process, but the proof is too long to be presented here. By accepting the translocation hypothesis, however, it has been possible to account for all the peculiarities of the oenotheras and to extend our genetic understanding of the genus much further. It is now possible, for example, to identify most of the chromosomes in the different complexes of many species and also to determine the location of many genes in these chromosomes. By identification of chromosomes is meant, rather, the identification of corresponding homologous regions in chromosomes of different constitutions. To do this, each chromosome end has been given a number from 1 to 14. In one complex, for example, there may be a chromosome in which end 1 is associated with end 2, while another complex has a chromosome in which end 1 is associated with end 4, as in Fig. 2, and so on.

In *Oe. Lamarckiana,* the *velans* complex is now known to be made up of chromosomes 1 · 2, 3 · 4, 5 · 8, 6 · 7, 9 · 10, 11 · 12 and 13 · 14. The *gaudens* complex is known to have chromosomes 1 · 2 and 5 · 6 with the remaining five chromosomes not yet completely identified. One of the remaining possibilities for these five chromosomes is 3 · 9, 4 · 12, 7 · 11, 8 · 14 and 10 · 13, and this will be accepted tentatively for the purposes of the present review. Chromosome 1 · 2 is present in both complexes of *Oe. Lamarckiana* and constitutes the chromosome pair in the diakinesis configuration in that species (see Fig. 1). The remaining six chromosomes of each complex are in the large ring and must occupy definite positions in relation to each other, as indicated in Fig. 3. Chromosome 1 · 2

FIG. 3. A diagram illustrating the exact relative positions in the large ring of *Oe. Lamarckiana* which the particular chromosomes of the *velans* (v) and *gaudens* (g) complexes must occupy because of the arrangements of corresponding end-segments in the chromosomes. The positions of certain characteristic genes are also indicated. In this diagram the ring of twelve chromosomes has been broken at "a" so that the diakinesis and anaphase configurations can both be inferred from a single two-dimensional figure.

sometimes carries the gene R, which produces red-veining of the leaves. When present, this gene segregates independently of the remainder of the two complexes. Chromosome 3 · 4 of *velans* carries the dominant gene P^s, producing red bud cones, in the 3-end of the chromosome. The recessive gene n, for *nanella* stature, is carried in the 4-end of chromosome 4 · 12 of *gaudens.* Further, there is a zygotic lethal carried in either 5 · 8 or 6 · 7 of *velans,* which prevents this complex from appearing in the homozygous condition. *Gaudens* similarly has its zygotic lethal carried by one of chromosomes 3 · 9, 4 · 12, 10 · 13; just which has not yet been determined. Except for genes

carried in the pairing chromosome 1 · 2, the genes characteristic of *velans* and *gaudens* must, as a rule, be cleanly separated by the mode of chromosome disjunction characteristic of the oenotheras (Fig. 3).

Origin of Derivative Types

Occasionally, crossing-over occurs in the 4-arm of chromosomes 3 · 4 and 4 · 12 between the locus of the gene n and the points in these chromosomes at which their homologies change. As indicated in the accompanying diagram (Fig. 4), a cross-over of this sort transfers the gene n from the 4-arm of chromosome 4 · 12 of *gaudens* to the 4-arm of chromosome 3 · 4 of *velans* without altering

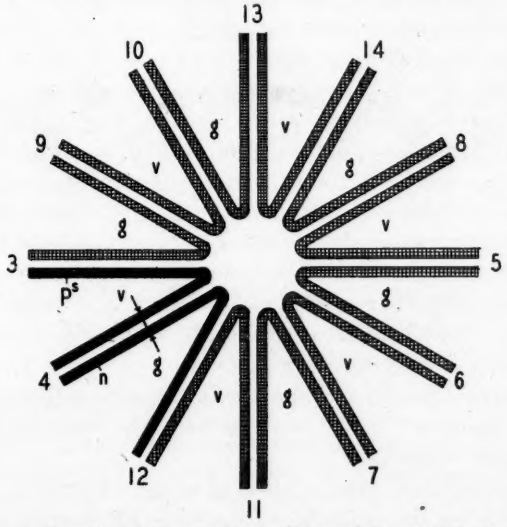

Fig. 4. A diagram of the prophase pairing of the ring chromosomes of *Oe. Lamarckiana* to illustrate how crossing-over in chromosome arm 4, proximal to the locus of n, transfers n from the *gaudens* complex (g) to the *velans* complex (v). The region of crossing-over is indicated by the arrows.

the homologies of either chromosome. In anaphase, this 3 · 4 chromosome carrying the recessive gene n passes to the same pole as the other *velans* chromosomes. There

results a complex called n-*velans*. From a union of n-*velans* with the normal *gaudens*, also carrying n, mutation *nanella* appears. This form is consequently a homozygous recessive (for the gene concerned), which appears only when crossing-over has separated n from the balanced zygotic lethal with which it is ordinarily associated, thus substantiating an early conclusion of Muller's based on the behavior of balanced lethals in Drosophila. This cross-over must occur in approximately 0.5 per cent. of all spore mother cells, as indicated by the frequency of appearance of mut. *nanella* shown in the table.

Mutation *rubrinervis* probably also arises by crossing-over, but the products in this instance are remarkably different from those in the case just described.[2] There is presumably a region near the middle of chromosome 13 · 14 of *velans* which is homologous to a region near the middle of chromosome 7 · 11 of *gaudens*. These regions are in chromosomes widely separated in the ring. If they can pair and cross-over, as indicated in Fig. 5, a whole series of changes will be initiated. In the first place, such crossing-over will not leave the end-homologies unaltered, as in the preceding example. Instead, chromosome end 7 of the *gaudens* chromosome 7 · 11 becomes associated with end 13 of the *velans* chromosome 13 · 14 to give a "new" chromosome, 7 · 13, which is unlike any of the chromosomes normally present in either parental complex. The other product of the same exchange is of course chromosome 11 · 14, another "new" chromosome but one with which we are not concerned.

In the second place, when the chromosomes become arranged on the spindle with the characteristic zigzag appearance (Fig. 5b), the new chromosomes must alter the positions of the remaining chromosomes in such a way that some of the *velans* chromosomes accompany certain *gaudens* chromosomes to one pole with a corresponding mixture of chromosomes also passing to the opposite pole.

[2] Mutation *rubrinervis* has not been shown to originate in the manner herein described, but the parallel to mut. *formosa* of *Oe. pratincola* is so complete that a similar origin is probable.

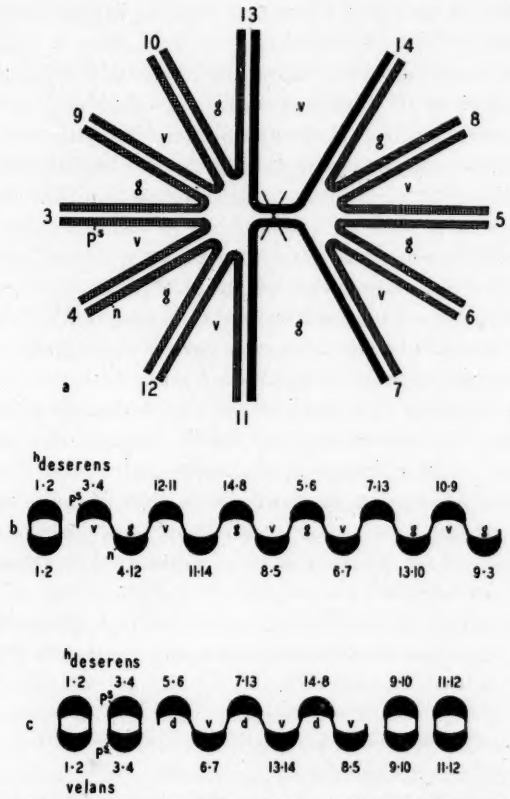

FIG. 5. Diagram illustrating the origin of the *hdeserens* complex which gives rise to mut. *rubrinervis;* a, prophase pairing with the region of crossing-over indicated by the ×; b, anaphase configuration showing the distribution of a mixture of *velans* (v) and *gaudens* (g) chromosomes and one new chromosome, 7 · 13, to one pole to form the *hdeserens* complex; c, the configuration of mut. *rubrinervis* (*velans* · *hdeserens*).

As a result, at one pole there is one member of the pair, 1 · 2, one new chromosome, 7 · 13, together with 3 · 4, 9 · 10 and 11 · 12 of *velans* and 5 · 6 and 8 · 14 of *gaudens*. These chromosomes make up a complex called haplo-*deserens*.

Mutation *rubrinervis* arises when a gamete carrying the *hdeserens* complex meets a normal *velans* gamete. This form (Fig. 5c) is homozygous for chromosome 3 · 4

and consequently for P^s, which results in the intensified red pigmentation characteristic of mut. *rubrinervis*. For the same reason, this form is homozygous for the normal allelomorph of n, thus explaining why de Vries never obtained mut. *nanella* from *rubrinervis*. Furthermore, the new complex, *'deserens*, has neither the zygotic lethal of *velans* nor that of *gaudens*. It obtained neither chromosome 5 · 8 nor 6 · 7 of *velans*, one of which carries the *velans* lethal, and it does not have chromosome 3 · 9, 4 · 12 or 10 · 13 of *gaudens*, one of which carries the lethal of that complex. This new complex, being entirely lethal free, appears in the homozygous form as a segregate from mut. *rubrinervis* and is known as mut. *deserens*.

The remaining types of derivatives observed by de Vries in his early experiments all have altered chromosome numbers. The changes in chromosome numbers responsible for the appearance of these forms were demonstrated by the cytological studies of Miss Lutz, Stomps, Gates and of de Vries and his students, in certain cases as early as 1907.

Mutation *gigas* was found to be a tetraploid having two complete sets of *gaudens* chromosomes and two complete sets of *velans* chromosomes. The exact origin of this form is not known, but it seems probable that the chromosome number has become doubled some time after fertilization. Except that there is greater irregularity in the disjunction of chromosomes in the tetraploid than in the diploid, accompanied by a greater amount of segregation of heterozygous characters, there is little that is peculiar about this form.

Mutation *semigigas*, found in the later studies of de Vries, proved to be a triploid. These triploid forms apparently arise from the union of one normal gamete and one unreduced gamete. Renner has recently shown that there are two recognizably different triploid types produced by *Oe. Lamarckiana*. In one there has been a union of an unreduced gamete with a *velans* gamete (*gaudens* · *velans* · *velans*), and in the other an unreduced gamete

has united with a *gaudens* gamete (*gaudens* · *gaudens* · *velans*).

Mutations *albida, oblonga, lata* and *scintillans* belong to a large group of trisomic forms which have one chromosome over and above the normal diploid number. Such trisomic derivatives are perhaps better known and understood in other organisms, but there are certain ways in which the trisomic derivatives of the oenotheras are noteworthy. In the progeny of *Oe. Lamarckiana* there is not only an unusually high frequency of trisomics, but these derivatives themselves represent many more types than are ordinarily produced directly from diploid forms. These two peculiarities are a direct consequence of irregularities in chromosome disjunction and in that way are related to the production of approximately 50 per cent. of "bad" or empty pollen grains.

It has already been noted that, as a rule, chromosomes lying adjacent to one another in the rings regularly pass to opposite poles of the spindle in the first meiotic division. In small rings with four or six chromosomes there are few or no exceptions to this rule, but in larger rings adjacent chromosomes occasionally pass together to the same pole. In the ring of *Oe. Lamarckiana,* such irregularities must occur in about half of the spore mother cells. Since there is always an even number of chromosomes in the rings, such irregularities can not occur singly; if two chromosomes in one part of the ring fail to separate normally there must be a compensating irregularity at some other point in the ring in order that the remaining chromosomes may disjoin in the regular zigzag manner. This situation can be seen from an examination of Fig. 6.

The relative positions in the ring of the two sets of irregularly disjoining chromosomes may vary to a considerable extent and give rise to quite different products. If one set of non-disjoining chromosomes passes to one pole and the other set to the other pole, as illustrated in Fig. 6a and 6b, the numerical distribution of chromosomes will be equal, but each daughter nucleus will entirely lack

some one chromosome arm. In the example illustrated (Fig. 6b), one nucleus is deficient for chromosome arm 5

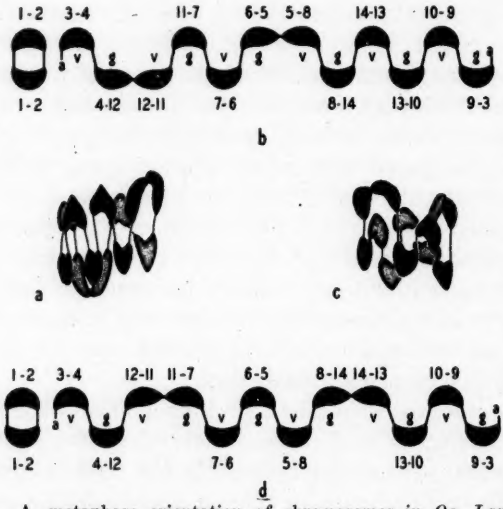

FIG. 6. A, metaphase orientation of chromosomes in *Oe. Lamarckiana* in which there are two irregularities which will result in an equal numerical distribution of chromosomes; b, a diagram of the same illustrating the qualitative differences between the chromosome sets passing to the two poles; c and d, instances in which the irregularities produce numerical inequalities, but in which the product receiving the larger number of chromosomes has each chromosome region represented at least once (a and c after Cleland).

and the other for chromosome arm 12. Spores which are deficient for any large part of any chromosome are known to be incapable of further development in plants, and irregularities of this sort in pollen mother cells must produce empty pollen grains.

In other instances both sets of irregularly disjoining chromosomes pass to the same pole, as illustrated in Fig. 6 (c and d). The products of such divisions will have six and eight chromosomes, respectively. The nuclei receiving six chromosomes will be deficient for two chromosome arms (arms 11 and 14 in the example illustrated) and will be incapable of producing functioning spores. The nuclei

receiving eight chromosomes, on the other hand, will have a complete set of chromosome arms and two arms in duplicate (arms 11 and 14 in the example illustrated). While pollen carrying such duplications is ordinarily inviable, these duplications are readily transmitted through the eggs and give rise to forms with extra chromosomes.

In the example illustrated, the nucleus receiving eight chromosomes has a mixture of *velans* and *gaudens* chromosomes (v and g, respectively, in the diagram). The exact number of *velans* and of *gaudens* chromosomes that will be incorporated in such cells depends upon the relative positions of the two irregularities in the ring. In all there should be thirty-six different 8-chromosome complexes arising in this manner from *Oe. Lamarckiana.* Six of these, however, must carry both the *velans* and *gaudens* zygotic lethals and the normal allelomorph of neither, and consequently can not function with either normal complex to produce a trisomic form. On the other hand, twelve other complexes will have the normal allelomorphs of both zygotic lethals and will function with either or both normal complexes to produce twenty-four different trisomic types. There should thus be a total of forty-two different trisomic derivatives directly obtainable for *Oe. Lamarckiana* as a result of the irregularities in the distribution of the ring chromosomes. A large number of these have already been obtained, but none has been studied sufficiently to indicate its exact chromosome make-up. It is known, however, that mut. *oblonga,* to name one example, is made up of one normal *velans* complex and another complex with eight chromosomes in which there is a mixture of *velans* and *gaudens* chromosomes. The constitution of this form indicates that it must have arisen in the manner just described.

Another peculiarity of the Oenothera trisomics is that while certain ones continually segregate into the trisomic and normal diploid forms, others breed true for the trisomic condition. If an 8-chromosome complex is made up of a complete set of *velans* chromosomes with the addition

of one *gaudens* chromosome and unites with a normal *gaudens* complex, there will result a trisomic form in which there is a complete set of chromosomes typical of the diploid *Lamarckiana* but with one *gaudens* chromosome in duplicate. There are various ways in which the chromosomes in such a form may be associated in meiosis, two of which are illustrated in Fig. 7 (a and b). Regular

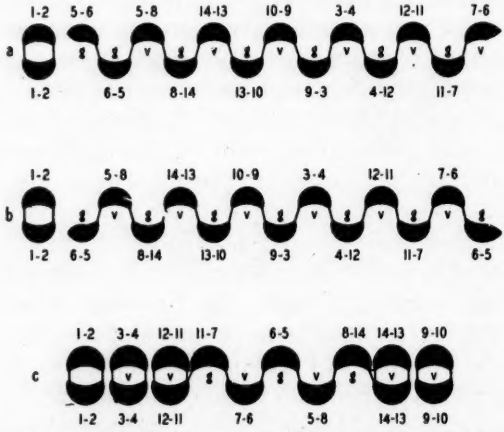

FIG. 7. Diagrams of regular chromosome disjunction in trisomic forms. That represented in a and b has a complete set of *Lamarckiana* chromosomes with 5 · 6 of *gaudens* as the extra chromosome. The distribution of chromosomes in this form may produce *velans* + 5 · 6 and *gaudens* (a) or *velans* and *gaudens* + 5 · 6. The trisomic represented in c is made up of a normal *velans* complex and an 8-chromosome complex in which there is a mixture of *velans* and *gaudens* chromosomes giving a net duplication for ends 11 and 14. In this form the configuration may vary between a chain of 9 and 3 pairs at one extreme and a chain of 5 and 5 pairs at the other, but normal disjunction for any of these must give the *velans* complex as one product and the mixed, 8-chromosome complex as the other.

anaphase separation following such associations will cause the extra chromosome to accompany the chromosomes of the *velans* complex in one case (a) and those of the *gaudens* complex in the other (b). A union of the normal *velans* and the normal *gaudens* complexes must give typical *Lamarckiana,* while a union of either 8-chromosome complex with the proper haploid complex will reproduce the trisomic form.

In other trisomics, however, in which the 8-chromosome complex is made up of a mixture of *velans* and *gaudens* elements, the association of chromosomes is always such that the extra chromosome accompanies the mixed complex. An example of this type is illustrated in Fig. 7c. In the example chosen there is a normal *velans* complex and an 8-chromosome, mixed complex. Whatever way the chromosomes become associated, normal anaphase separation can yield these two complexes and no others. Consequently such trisomic forms must breed true.

Irregular chromosome disjunction is to be expected in the trisomic forms as well as in the diploid. In the trisomics, however, there is usually a large chain of chromosomes instead of a closed ring and there may often be but one set of irregularly disjoining chromosomes. Such irregularities should still result in complexes with seven and eight chromosomes, respectively, but the 8-chromosome complexes will be composed of different elements than those of the original, 8-chromosome, parental complex. Hence it can be understood why there are always a large number of "secondary" trisomic types in the progeny of all "primary" trisomics, whether or not these "primaries" breed true for the trisomic condition.

It is now apparent that all the early "mutations" observed by de Vries in his controlled cultures arose through rearrangements of genetic elements already present in the parent form. Mutation *brevistylis,* one of the earliest variants studied by de Vries, undoubtedly arose as a true mutation shortly before he found it growing in the wild. It never appeared as a mutation in his controlled cultures, but since that time many other true mutations have occurred in inbred lines. Among these true mutations appearing in controlled cultures are the *rubricalyx* of Gates, *vetaurea, supplena, bullata* and others of Shull, and *angustifolia* and *stenophylla* of de Vries. In contradistinction to the types discussed above, these mutations represent actual changes of single genes to states not represented in the parental form.

GEOGRAPHIC ELEMENTS OF THE MARINE FLORA OF THE NORTH PACIFIC OCEAN[1]

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IN attempting to estimate possible origin and spread of land floras, it seems necessary, or at least very profitable, to analyze the composition of each and every particular flora into its possible geographic elements. Such entities, delimited as exactly as possible, may then be considered as to whether each individual element may have been of more recent or of more ancient introduction and, in this way, more or less tenable hypotheses may be suggested and supported both as to possible origin and as to time, manner and direction of invasion of the areas where now it is to be found. In discussing insular land floras, particularly those of oceanic islands, it seems almost incumbent to assume continental origins, in general, with spread to, and with possibly some later differentiation on, the islands themselves. Some students estimate the latter of much more considerable importance than do others. A more recent point of considerable agreement regarding certain of the more striking endemic elements of the floras of the oceanic Pacific islands tends to assume a Tertiary Antarctic continental origin with northward migration and establishment, not only among the higher islands of both Pacific and south Atlantic but also along the high lands of at least the southern continental masses.

In attempting to explain "presence" in cases of pronounced endemism, invasion *vs.* local evolution must necessarily be considered, but here again the matter is relative, since invasion of the ancestors, like or unlike, must necessarily prove a factor in the discussion, and

[1] Presented to the "Symposium: The Origin and Development of North Pacific Floras," joint meeting of Section G and the Botanical Society of America, American Association for the Advancement of Science, Berkeley, June 21, 1934.

must be influenced not only by the particular case, but also, by inference, from all other cases of similar type or types. In following out the idea of "invasion" too much stress has undoubtedly been laid on possibility of migration, as restricted to the mere travel of germules from the place of production to the place of coming to rest, and too little emphasis on the provision of opportunities of establishment, including persistence of the infinitesimally small number of migrant germules, the evidence of which is "presence." "Discontinuous distribution" is a term usually applied only to wide segregations of individuals of taxonomic groups. In reality, all distribution, even within the area ordinarily occupied by a species, is discontinuous and is, at times, fairly widely spaced. The explanation that this is due, not to lack of power to migrate, but to lack of ability to effect establishment, is the usual one. Control by environmental differences in such cases is readily understood, but this same explanation must also hold for intermediate unoccupied regions in most, if not in all, cases of distribution which is strikingly discontinuous. Climatic, edaphic and biotic factors control establishment; all three complexes of extreme diversity, while migration, in the strict sense, depends chiefly upon the effective operation of agency and device facilitating spread of germule, although interposition of opportunity for starting on the journey is often very critical, as well as its reiteration. Travel over land may be deemed possible for all members of a land flora, but this is possibly more supposititious than real. For aquatic plants, freedom of water movement may be deemed even more essential, but any extreme cloture of possibility of travel over barriers of land is not, by any means, to be assumed.

Aquatic plants may be roughly divided among the attached, or benthos; the larger plants of normally floating habits, the pleuston; and the microscopic floating plants, the plankton. It seems best to restrict our discussion of marine flora to the benthos constituent, having in mind

that many, if not all of its members may have pseudopleuston stages, in which, becoming detached, they float and may even carry, unharmed, their germules for long distances. The marine benthos is very satisfactorily divided between the Sea Grasses and the Seaweeds, according as to whether they are seed plants or spore plants; the former, of course, being of much more complex structure than the latter.

The Sea Grasses are all monocotyledons of the order Fluviales or Helobiae, an aquatic group of simpler and possibly more primitive type of flower structure than the rest of the monocots. The marine members of the Fluviales are distributed through two families, the Hydrocharitaceae and the Potamogetonaceae, but constitute various tribes or subfamilies of these, most of which, in turn, may possibly better be considered as independent family groups. The marine members seem likely to be descended from certain fresh-water lacustrine or fluviatile ancestors of the two families which have been able to adapt themselves to a halophyte existence. These possibilities and probabilities are to be borne in mind in any discussion of origin and spread.

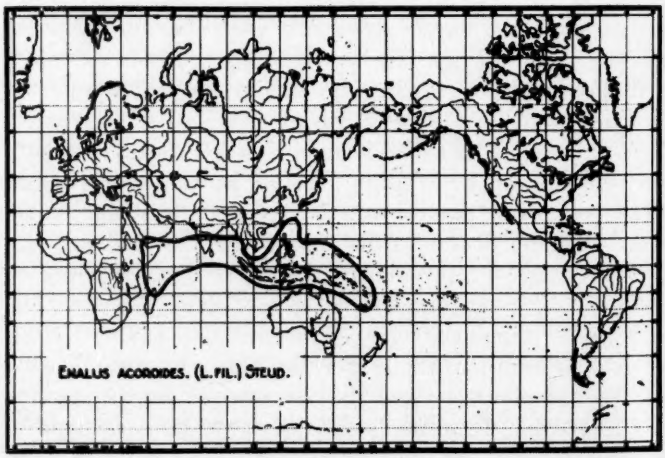


FIG. 1

The marine members of the Hydrocharitaceae are practically tropical in their distribution. Only two species extend beyond strictly tropical waters and it may be presumed that this extension may be due to seasonal adaptation. Of the twelve species of *Enalus* (see Fig. 1), *Thalassia* and *Halophila* concerned, eight are confined to the Indo-Pacific region (two only extending out eastward into the Pacific Ocean proper) while four are characteristic of the eastern Caribbean region of the Atlantic. The detail of their distribution is well shown by Ostenfeld (in "Die Pflanzenareale," 1 Reihe, Heft 3, 1926), who discusses further the presence of vicarious pairs of species (*i.e.*, two species only slightly discontinuous in structural differences but widely discontinuous in distributional segregation), between the Indo-Pacific member of the pair and the Caribbean member. One such pair (see Fig. 2) is constituted by *Thalassia Hemprichii*

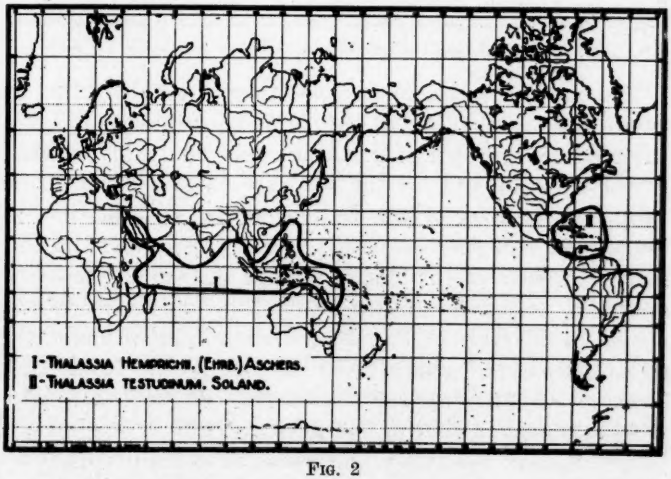


FIG. 2

(Indian Ocean) and *T. testudinum* (Caribbean Sea), the latter found also on the Pacific Ocean side of the Isthmus of Panama, but whether attached or established and whether of recent or ancient occurrence is uncertain.

Another vicarious pair is represented by *Halophila decipiens* (Indo-Pacific) and *H. Baillonis* (Caribbean) (see Fig. 3).

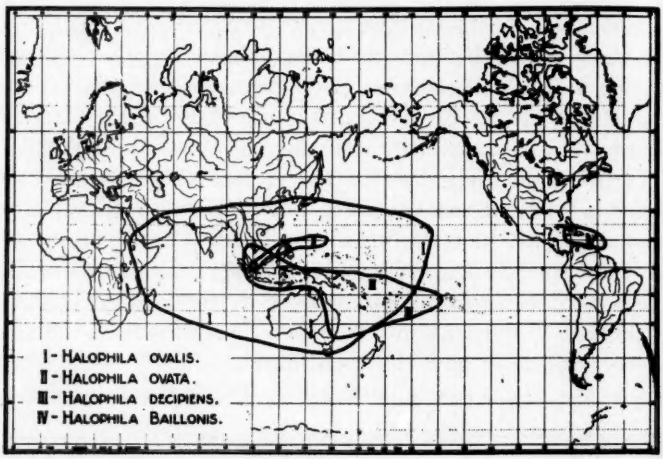


FIG. 3

The marine Hydrocharitaceae, then, are chiefly Indian Ocean plants, tropical, in nature and presumable origin, entering the north Pacific Ocean only sparingly in central Polynesia and on the eastern Asiatic coast, both simply extensions of the Indian central distribution of the group. No species of this family is known from the American side of the Pacific except the single, possibly sporadic, occurrence of the Caribbean *Thalassia testudinum* in the Gulf of Panama. Since, in all probability, members of this group occur in among the fossil Sea Grasses of the upper Cretaceous of eastern Asia (Japan?) and in that of the ancient Tethys, origin may be assumed in the latter and outward spread from it as a center. No marine record of any recent species of this group in the Mediterranean regions is available except that *Halophila stipulacea* (Red Sea and east Africa), has been found at Rhodes, but Ostenfeld (*loc. cit.*, p. 38), seemingly justifiably, regards this occurrence as a pos-

sible ship-waif. It is also significant that *Halophila Aschersonii,* member of a distinctive Caribbean vicarious pair of species, has been recorded (ship-waif?) as far south as Pernambuco, Brazil (see Fig. 4).

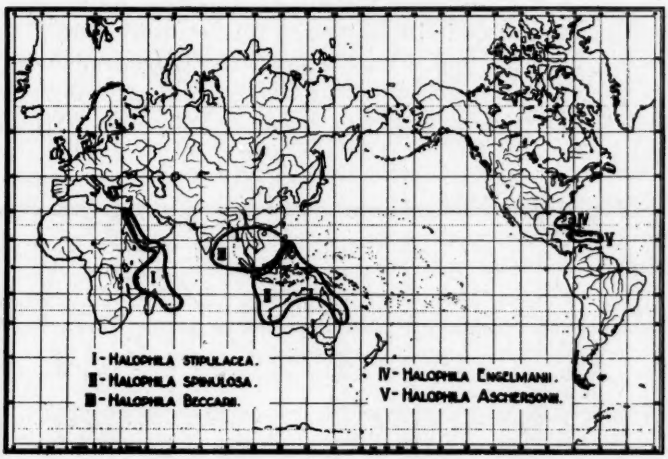


FIG. 4

If the center of possible origin and spread may be thought of as eastern Tethys, the Caribbean group, because of the presence of members of vicarious pairs of species, may be attributed to Indo-Pacific source and indicate some earlier (*i.e.,* earlier than the present or even late Tertiary) broad Pacific distribution, at least of migration. The spotwise distribution of one species in the Mediterranean, another species in the Gulf of Panama and a third at Pernambuco, Brazil, may also signify powers of travel or migration-efficiency, but possibly, or even probably, without a corresponding adaptability towards establishment.

The marine Potamogetonaceae constitute three of the five very distinct subdivisions of the family, the other two, the Zannichellieae and the Potamogetoneae, being largely of fresh-water habitats, but with minor representation in (or intrusion into) brackish waters. Of the

marine members, the Cymodoceae, with two genera, are largely tropical, but with one species of Cymodocea in the subtropical waters of the Mediterranean and adjacent Atlantic coasts: the Posidonieae, with a single genus of two species of subtropical to temperate waters and antipodally discontinuous, one in the Mediterranean, the other in extratropical Australian waters: while the Zostereae (or Zosteraceae?), with two genera, are subtropical or temperate, with one species of decidedly frigid tolerance.

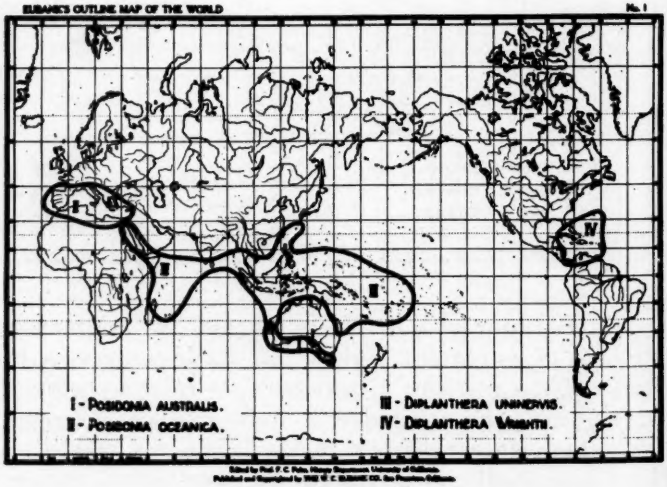


FIG. 5

Of the two species of *Posidonia,* a vicarious pair, neither enters the north Pacific Ocean, the one *P. oceanica,* being almost confined to the Mediterranean (with an outlier in the Bay of Biscay), the other confined to the southern and southwestern coasts of Australia. If Tethysian in origin, temperature requirements and temperature tolerance are possibly derivative rather than primitive and of crucial importance in establishment rather than in migration.

Of the two genera of Cymodoceae, *Diplanthera* is rep-

resented by three species, of which *D. uninervis* and *D. Wrightii* form a closely related vicarious pair (see Fig. 5), the former Indo-Pacific, intruding slightly into the north Pacific in the Austral-Asian region, and the latter Caribbean (with an outlying record in the Gulf of Panama). The third species, *Diplanthera pinifolia* Miki, very recently described (1932), is confined to the Riukiu Islands of Japan and is closely related to *D. uninervis.*

The other genus of the Cymodoceae, *Cymodocea,* is divided into three subgenera, *Phycagrostis,* with three species, *Phycoschoenus,* with two species (both subgenera very closely related to *Diplanthera*), and *Amphibolus,* with three species, less closely related to *Diplanthera,* but with one species intermediate between *Amphibolus* and *Phycagrostis.*

Of the subgenus *Phycagrostis,* one species (*Cymodocea angustata* Ostenf.) is known, as yet, only from a single

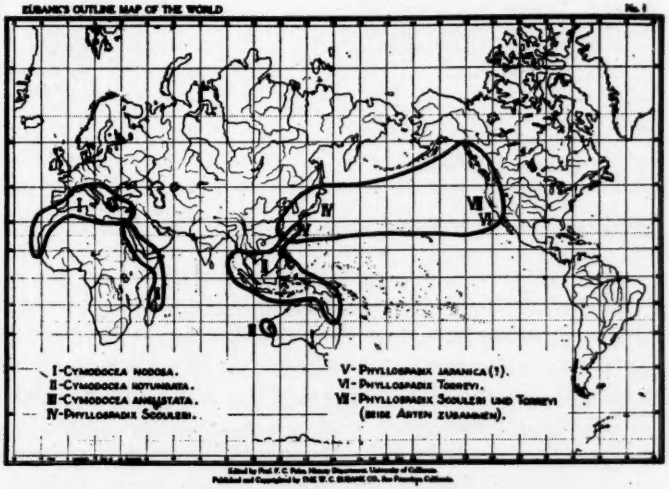


FIG. 6

locality in west Australia. It is an intermediate species, whose origin may possibly be hybrid. Of the other two species of *Phycagrostis, Cymodocea rotundata* is chiefly

of Red Sea occurrence, but enters the north Pacific, proceeding up to the Riukiu Islands (Miki, 1932), while *C. nodosa* is Mediterranean, passing out into the Atlantic to southwest Spain and through the Canary Islands to Senegal (see Fig. 6).

Of the subgenus *Phycoschoenus,* with cylindrical leaves, the two species form a vicarious pair, *C. isoetifolia* Indo-Pacific, entering the north Pacific on the southwest (up to the Riukiu Islands) and *C. manatorum,* Caribbean (see Fig. 7).

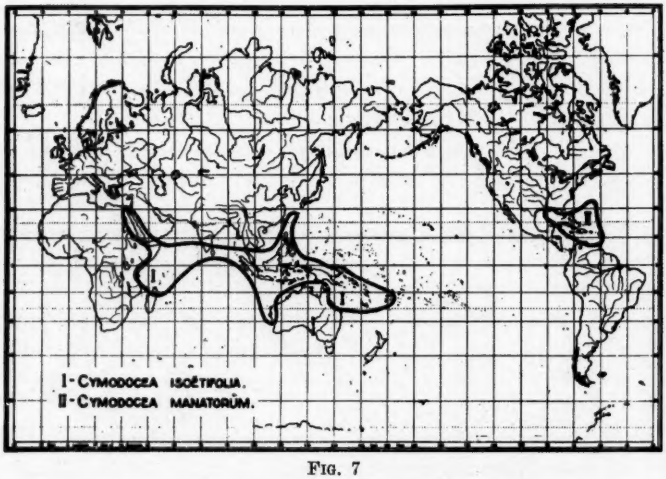


FIG. 7

Of the third subgenus, *Amphibolus,* one species, *Cymodocea antarctica,* is so distinctive as to be assigned by some writers to a separate genus. It is a plant of extratropical Australian shores, showing the same range as *Posidonia australis* (see Fig. 8). *Cymodocea ciliata* and *C. serrulata* are both Indo-Pacific, the former discontinuous as to East Indian and West Indian areas, while the latter is continuous from West to East Indian and the northwest Pacific to the Riukiu Islands (see Fig. 8).

The north Pacific relations of the Sea Grasses thus far indicated seem to be confined to a sparse overflow, or

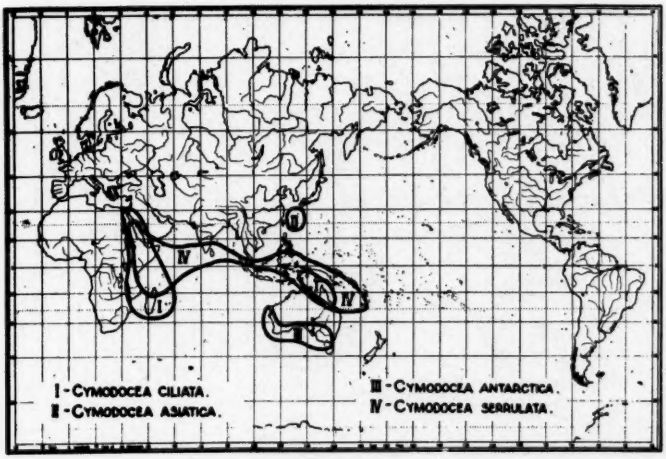


FIG. 8

outspread, from an Indian Ocean center to the central Pacific and to the warmer waters of the eastern Asiatic coast, with a Tethysian origin being seemingly the most suggestive.

The Zosteraceae, or Zostereae, of the Potamogetonaceae have no strictly tropical representatives. They may be compared rather with the species of the Posidonieae (*Posidonia oceanica* and *P. australis*). In this, they may be connected, but less definitely, of themselves, with Tethysian origin and oceanic spread. Of the two genera concerned, *Zostera* seems the more primitive, at least as regards the species of the subgenus *Zosterella,* while *Phyllospadix* seems to be made up possibly of ecospecies, at least the mechanical, vegetative elements are definitely adaptive and much exaggerated over those of *Zosterella.* This seems to be a matter of size and form, with relation to environmental specialization.

Of the six species of the *Zosterella* section, as limited (Setchell, 1933), two areas of distribution seem marked; the Mediterranean region and the extra-tropical Australian region (see Fig. 9). *Zostera nana* of the Mediterranean (and adjacent Atlantic coasts) has vicarious

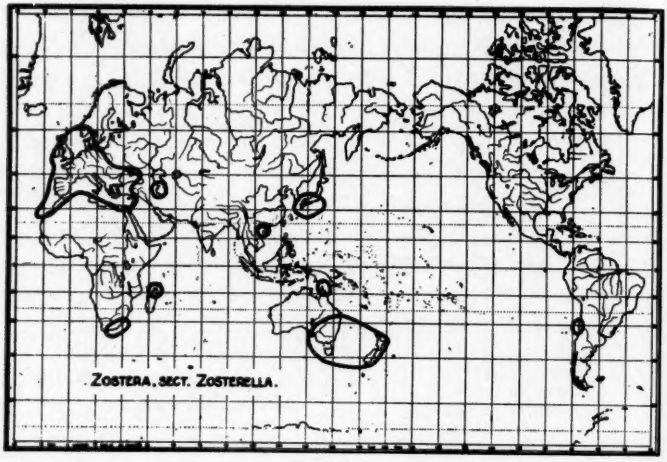


FIG. 9

multiples in South Africa, east Australia, New Zealand and the extra-tropical portion of the north Pacific in Japan. All the species of *Zosterella* have spadices of a simple type, with well-developed bracts, or "retinacula." The single species belonging to the subgenus *Heterozostera* (*Zostera tasmanica*) departs from the more simple *Zosterella* structures in greater development of mechanical and conducting tissues and to a slight extent points towards the greater development of such structural elements in *Phyllospadix*. Its home is in southeastern Australia, with an outlying station on the coast of Chili.

The distinctive Sea Grasses of the North Pacific belong to the genus *Phyllospadix* and are restricted to temperate waters, with little frigid tolerance. Ostenfeld (Roy. Soc. Victoria, N. S. 27 (2): 190, 1915) considers them to have been derived from *Zostera* (*Zosterella*). The distribution of the species (see Fig. 6) is confined to the western coast of North America and the Japanese coasts of eastern Asia. There are possibly three species on the North American coast and two on the Japanese coasts (Miki, 1933). The species form a distinct geo-

graphic element in themselves, probably of ancient (Cretaceous?) but possibly of more recent (Tertiary?) development.

Finally, among the Sea Grasses there may be discussed the species of the subgenus *Alega* (see Fig. 10) of the

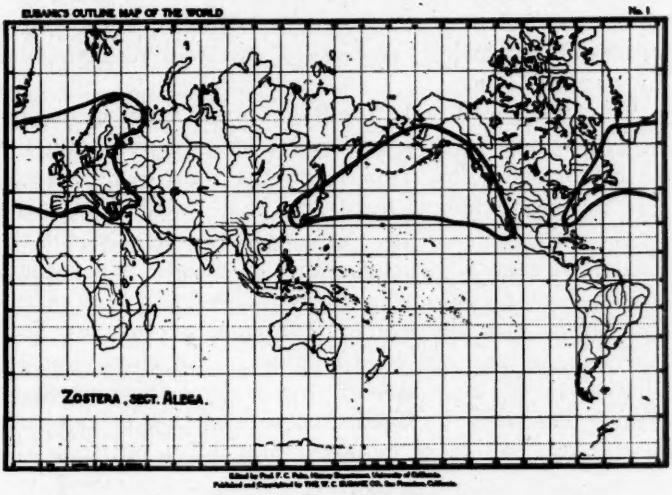


FIG. 10

genus *Zostera*. The long-known type species of this subgenus, *Zostera marina*, or common Eel Grass, has a wide distribution in the northern portions of both the Pacific and the Atlantic oceans. While presumably an ancient form, it seems to have lost, completely or almost, the characteristic bracts, or retinacula, of the seemingly less modified species of the *Zosterella* and *Heterozostera* sections of *Zostera* and of *Phyllospadix*. Very recently Miki (1932) has carefully studied the Sea Grasses of Japan and from numerous collections he not only confirms *Zostera marina* for Japan, but adds three additional well-marked but closely related species. Since Koriba and Miki (1931) also find abundant Japanese fossils of some seven species of what they have named *Archaeozostera*, belonging to the Upper Cretaceous, and

which they hold to be the ancestors of *Zostera* (Miki, *The Botanical Magazine of Tokyo,* 46: 774, December, 1932), there may be some reason for considering that the *Alega* species originated in the north Pacific region, or possibly migrated from a northern extension of Tethys. A similar migration from Tethys may account for the presence of *Zostera* in the north Atlantic, since it also occurs in the northern portions of the Mediterranean and in the Black Sea.

To summarize briefly, the Sea Grasses may be roughly segregated as to distribution into the tropical and the extratropical. The tropical groups represent, at present, two geographic elements, the Indo-Pacific (really the Indian) element and the Caribbean. Because of the existence of several vicarious pairs of species, or geminate species, one member of each pair present in each of the two areas now segregated from one another by land barriers, it may not be assuming too much to suggest that these two geographic elements come from the same source and, since the Indian source seems the more representative and lies at, or near, the eastern outlet of the Cretaceous and early Tertiary Tethys, that these two geographic elements are really Tethysian, possibly of earlier wider north (as well as south) Pacific representation but now confined to those two areas which present most nearly the environmental conditions of the Tethys of Cretaceous and earlier times, *viz.,* the East Indian shallow seas and the Caribbean Sea.

The extratropical species present in their present distribution both supporting and antagonistic arguments. These Sea Grasses tend to distribute themselves between two antipodally discontinuous areas of present distribution, *viz.,* Mediterranean and Australian. Ostenfeld (1915, p. 190) thinks that some of these at least originated from tropical forms, or at least like the two species of *Posidonia,* they have been dispersed northwards and southwards, respectively, from an earlier Indian Ocean habitat. Even in the case of the species of the *Alega*

section of *Zostera,* and of those of *Phyllospadix,* probably independently evolved through some warmer water species of the *Zosterella* section, this may also hold. The north Pacific elements, then, so far as Sea Grasses are concerned, may be Tethysian, possibly of Indian and Pacific dispersal, although the extratropical species may possibly be of boreal transmission.

The Seaweeds are much less compact as a group, or even as subgroups, and while they present certain indications of Tethysian origin and oceanic dispersal, present also cases of more probable boreal or austral origin. Svedelius (Arkiv för Botanik, 19: No. 3, 1924) has given many important details of general and vicarious distribution for this constituent of the marine flora and has been constantly referred to for data.

Among the Blue Green Algae distribution of species is wide-spread, but such genera as *Brachytrichia* and *Hormothamnion* repeat the Indo-Pacific-Caribbean balance, the former genus, in fact, extending it to the Mediterranean region.

Among the Green Algae most genera and many species are almost cosmopolitan. Among the tropical species of calcareous genera such as those of *Halimeda* and the verticillate Siphoneae, the Mediterranean-Indo-Pacific-Caribbean distribution extends to genera, as well as for some species. The calcareous forms, favorable for fossil preservation, are represented as early as Ordovician and Silurian times, and in many genera not now persisting. They were abundant in early Tethys.

For the Brown Algae, the geological series is both most imperfect and obscure, presumably because of the very perishable nature of their plant bodies. Three groups of their modern representatives show present distribution of particular interest and involving the north Pacific Ocean. These groups are the Gulf-weeds or Sargassums, the Rock-weeds or Fucaceae and the Kelps or Laminariales.

Sargassum is a genus of somewhat over two hundred

accredited species. It is tropical and subtropical of all larger seas and oceans. It is usually divided, according to vegetative structure, into five subgenera. Of these,

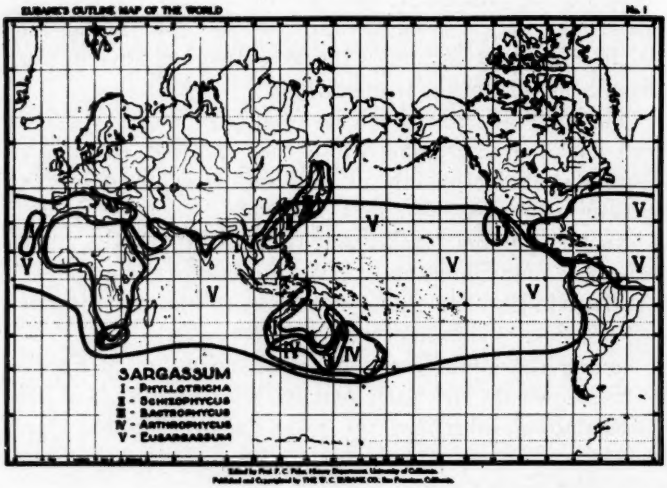


FIG. 11

subgenus V, *Eusargassum,* seemingly the most complex, contains much the largest number of species (about two thirds of the whole number) and has the widest distribution. We may, however, segregate the species of *Eusargassum* imperfectly into Caribbean, Mediterranean, Indian and Pacific elements, with possible Tethysian centers of origin. Of the other four subgenera, one, *Arthrophycus* (see Fig. 11), is exclusively austral (South African and Australian), while two are boreal, *viz.,* II, *Schizophycus,* and III, *Bactrophycus* (see Fig. 11). The center of the distribution of these two is northeast Pacific, from Hong Kong to northern Japan. Subgenus I, *Phyllotricha* (see Fig. 11), tends somewhat to repeat the *Posidonia* and *Zosterella* distribution. Its main occurrence is tropical and extratropical Australian, with extension along the western edge of the Pacific towards Japan, where it may be represented also by the

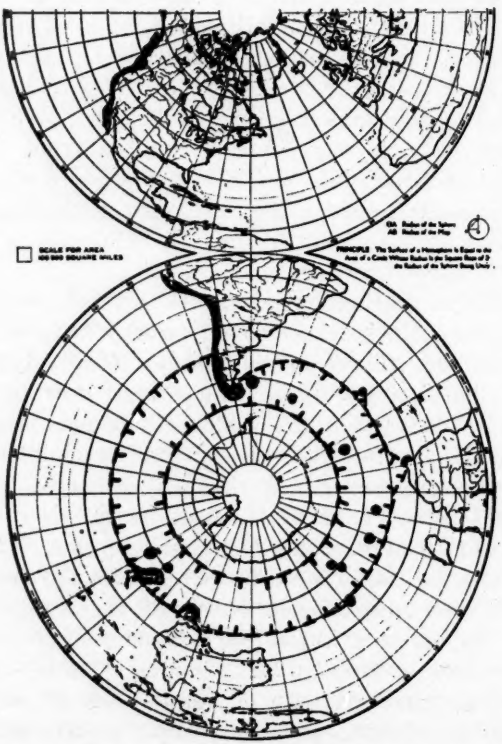
single member of subgenus II, *Schizophycus*. There are, however, two outlying species of *Phyllotricha;* one, *Sargassum comosum,* in the Canary Islands, the other, *S. Palmeri,* in the Gulf of California and Guadalupe Island. We have in the distribution of these extratropical Sargassums a reminder of the distribution of the Zosteraceae, only less perfect, but possibly suggestive of similar origin and dispersal. The Sargassaceae other than *Sargassum* follow much of the distributional peculiarities of the Sea Grasses, there being a distinct austral group of some size and complexity, a tropical Indo-Pacific-Caribbean group, and a Mediterranean group, but there is also a distinct north Pacific group, as there is in the Kelps, as well as in the Zosteraceae.

The Rockweeds, or Fucaceae, are a largely boreal group with slight austral representation. There is sufficient, however, to suggest a derivation from warmer water forms proceeding each both north and south. The family is more considerably developed in the north Atlantic than in the north Pacific. If Tethysian in origin, it follows the Zosteraceae pattern.

The Kelps, or Laminariales, cold to fairly warm temperate forms, include somewhere about five fairly distinct families. Three of the simpler family types are boreal, the simplest of all (Chordaceae) being represented best in the north Atlantic but also occurring sparingly in the north Pacific. Another simple family (Haligeniaceae) centers in the Mediterranean and adjacent Atlantic. A third (Laminariaceae) is distinctly boreal and cool temperate, being well represented in the north Atlantic, but more differentiated in the north Pacific. It has, however, one outlier in the southeast Atlantic, *viz., Laminaria pallida,* abundant in the vicinity of Cape Town. This outlier, presumably, shows greater powers of migration than of adaptability of establishment for the group. The fourth family (Alariaceae) is represented by one tribe (Alarieae) in both north Pacific and north Atlantic, possibly by greater complexity in the

north Pacific, while another tribe (the Ecklonieae) is austral, reappearing, however, in a surprising variety of genera and species on both sides of the extratropical north Pacific. The last family (the Lessoniaceae) encircles the Antarctic region, proceeds up the west coast of South America, skips the tropics and then, from Lower California up, occupies the western coast of North America and passes to the Sea of Ochotsk on the Asiatic side (see Fig. 12 for distribution of *Macrocystis*).

This mingling of boreal and austral forms of Kelps, in the north Pacific, seems difficult of interpretation with



Adapted from Map No. 101P of the Goode Series of Base Maps and Graphs, by permission of the University of Chicago Press.

FIG. 12

our present data. They seem more likely to have been of oceanic origin than Tethysian, although the seemingly more primitive Mediterranean Haligeniaceae may still point in that direction.

The Red Algae of the tropical Pacific, both north and south, show in general similar relations to those shown by the other groups. Tropical genera, while found in all oceans, show pronouncedly Indo-Pacific-Caribbean relations and many vicarious pairs of species. The calcareous Melobesieae are especially noteworthy for such vicarious pairs. For extratropical genera, Mediterranean, austral and boreal types are usually different, but some genera have close relatives in every one, and especially there is a distinct austral (American) element in the north Pacific.

It is certainly a great temptation, when passing in review the details, past and present, of the distribution of our benthic marine floras, to hark back to the very ancient Greek idea that Tethys is the mother and Oceanus the father of all things. Undoubtedly Tethys had much to do with origins and the oceans much to do with dispersing, but adaptation, yielding establishment and persistence, may play so important a part as to make obscure much of past history. It must be borne in mind that the characteristic conditions of tropical Tethys are now most pronounced in the north Indian Ocean (especially in the Sunda and adjacent seas) and in the Caribbean region while extratropical Tethys conditions were probably more general.

PLETHODON CINEREUS (GREEN) IN EASTERN PENNSYLVANIA AND NEW JERSEY

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BECAUSE of its abundance and wide-spread distribution, the natural history of the red-backed salamander has received considerable attention. With the exception of the general summary of Dunn (1926), the best papers discuss *P. cinereus* in the northern part of its range: Piersol (1910, 1914) at Toronto, Canada; Cochran (1911) in New England; Blanchard (1928) for Michigan. In addition, numerous briefer accounts exist from that of Cope (1889) to the present time.

The data presented here have been derived from my collections made during the past five years in eastern Pennsylvania and New Jersey and, in particular, from over one thousand red-backs, taken weekly during 1934 from localities spaced between Allentown, Pennsylvania, and Princeton, New Jersey. Hence, there is offered a numerical survey of this area, together with new facts, which may be of interest in filling out our knowledge of the natural history of this species.

I wish to thank Dr. E. R. Dunn for the use of some of his unpublished records, and for pointing out that the literature does not contain a statement that both color phases are found in the same clutch of eggs, which is a clear demonstration that the two phases are of the same species. Also, I am indebted to Dr. E. G. Butler for assistance in the preparation of the manuscript.

DISTRIBUTION AND HABITAT

P. cinereus is widely and abundantly distributed over eastern Pennsylvania and northern New Jersey. In Bethlehem, Pennsylvania, it was found on lawns well within the city limits. However, it is not so abundant on the Coastal Plain of New Jersey. It has been found

along the Delaware River as far south as Cape May (Dunn, 1926). Specimens have been collected at New Lisbon, New Jersey (Dunn, unpublished), at the edge of the Pine Barrens, and I have found a few individuals at Chiselhurst, New Jersey, which is situated within the Pine Barrens. At Princeton, I have been unable to collect it east of Lake Carnegie, which lies on the boundary between the Coastal Plain and the Triassic Lowlands. On the west side of Lake Carnegie, however, the red-backs are numerous.

One feature concerning the distribution of *P. cinereus,* which has not been mentioned in previous accounts, is the occurrence of densely populated separated loci of habitation. A small area of about 600 square yards may contain a hundred or more red-backs. Between this situation and another, *P. cinereus* may be absent for distances up to a mile, even though the forest vegetation is uniform. On one occasion a small woods of about two acres in area was thoroughly worked over. Only ten red-backs were collected, and all were found within a radius of ten feet. In favorable situations, on the other hand, the overturning of almost every stone will reveal a red-backed salamander. It should be mentioned, furthermore, that in collecting *P. cinereus,* one is not so likely to skim the surface of the population, as Kline and Fuller (1932) found to be the case with Desmognathus. However, it would be misleading, if it were not made clear that distribution is often over a wider area than has been described, and that the population is often uniformly scattered over such areas.

By understanding the habitat requirements, some of the reasons for irregularities in the distribution of *P. cinereus* may be clarified. The most favorable situation is a forest floor, not heavily covered with underbrush, with a good clay bottom and provided with suitable shelter in the nature of logs and stones. Permanently wet and boggy places are not populated. After a rain, the salamanders will come to the surface to avoid the

accumulation of water in the ground. Their tolerance of dryness is much greater than that of aquatic forms. I have subjected *P. cinereus* together with *Eurycea bislineata* and *Desmognathus fuscus* to winter weather for a week, with no protection but dry leaves. After this exposure some of the Plethodons were still alive, while the other two genera were desiccated and dead.

Throughout the area in which collections have been made, stones are more frequently used for cover than are logs. But too many stones and too little soil, with a resulting lack of food, appear to be the most important reasons for an interrupted range in a forest. Unfavorable situations are very common. Situations which are entirely favorable exist in restricted areas and, therefore, tend to concentrate the population.

Mingled with *P. cinereus* one finds *Plethodon glutinosus, Hemidactylium scutatum,* the land stage of *Triturus viridescens, Ambystoma maculatum* and occasionally *Pseudotriton ruber*[1] and *Eurycea bislineata.*

SIZE AND COLOR PHASES

The average adult body length, exclusive of the tail, is 41 mm for the female, with size limits of 38 to 48 mm. The males average 39 mm, with limits of 34 to 46 mm. Specimens were preserved in 80 per cent. alcohol. Curves similar to those of Blanchard put the maxima at 41 mm for the female and at 40 mm for the male.

Blanchard's treatment of size groups appears quite adequate. However, it would be interesting if reliable data could be assembled in regard to the percentage of larvae which reach sexual maturity. In the case of Plethodon a remarkably favorable start in life is assured by the circumstances that only a few eggs are laid, each egg is well supplied with yolk and the female remains with the eggs until after the larvae are hatched. Nevertheless, great variability exists in regard to the number

[1] It was noticed that even in dry weather *P. ruber* was found far from its customary habitat, probably left stranded after a night's hunting.

of young which mature. In certain circumscribed areas, a great preponderance of adults is found. In other areas, adults are less numerous than are the young. The reasons for this variation are not entirely clear, but are probably associated with the problem of food supply.

The costal groove number is generally 18, plus the two over the shoulder. The last groove anterior to the hind leg is frequently forked. Dunn has found no geographical variation in regard to the number of grooves, a condition which I have found to hold true in the region where I have collected.

So far as I am aware from an examination of the literature, no one has been able to demonstrate conclusively that the two color phases of *Plethodon cinereus* are the same species. Cope (1889, p. 136) states, "As varieties they are very permanent ones, as I have found all the young of the same brood or set of eggs, whether in the eggs or just escaped from them, uniformly with either dark backs or red ones." Neither Piersol (1910, 1914) nor Cochran (1911), both of whom found eggs, refute this statement, although the poor photograph of four individuals from the same clutch of eggs, which Cochran has used, appears to show two red-backs and two black-backs. I have repeatedly found both phases in the same set of eggs, which conclusively proves that the two color phases are the same species and come from the same parents. Unfortunately, I have not collected enough egg clutches to make a justifiable statement with regard to the ratio in the individual clutches. In part, Cope may have been misled by the fact that the back of the black phase is lighter during development than after hatching. The data in regard to the color phases of the adult animals, which I have collected, will be found in Table 1.

The 1:3 ratio of the red phase to the black phase found at Princeton appears to be purely a local matter. A collection (not included in Table 1) made a few miles west of Princeton gave a 1:1 ratio. The evidence shown

TABLE 1

Locality	Number of red phase	Number of black phase	Ratio
1. Allentown, Pennsylvania	133	139	1:1
2. Hellertown, Pennsylvania	53	34	1:1?
3. Perkasie, Pennsylvania	154	175	1:1
4. New Hope, Pennsylvania (west bank of the Delaware River).	55	43	1:1
5. New Jersey bank of the Delaware River, opposite New Hope, Pennsylvania	47	53	1:1
6. Princeton, New Jersey	36	110	1:3
Total number collected	478	554	

in Table 1 indicates that the Delaware River has exercised no segregating influence. There is only one questionable case (Hellertown, Pennsylvania) of red-backs being more numerous than black-backs. In this case the total number collected is too small to draw definite conclusions. Recently Hood (1934) has found 95.7 per cent. red-backs at Genesee Gorge, Rochester, New York. Cope (1889) on the statement of Baird records nothing but red-backs on the west side of Lake Champlain, in Essex County, New York. Dunn (unpublished) found none but red-backs at Mt. Lake, Charles County, Virginia, at an elevation of 4,000 feet. He records (1926) a predominance of red-backs at Linville, North Carolina. Breder and Breder (cited from Dunn, 1926, p. 167), on the other hand, found the black phase more dominant at Beaver Creek, Ashe County, forty miles away. But in the main, the ratio of 1:1 appears to prevail throughout the East.

No unusual colorations have been found, except a tendency to red-headedness for the Hellertown animals. While not a constant character, it is marked in a number of animals. It may be pointed out that while the adult coloration is by no means uniform, especially in the red phase, the pattern of the young larvae is clear and distinct.

Habits

P. cinereus is almost solitary. I have never found more than four together under one object during warm weather. With snow on the ground, I have found them alone under deeply imbedded logs. However, congregations sometimes are found. On April 5, at Princeton, twenty-three individuals of all sizes were found together in a sluggish condition under one stone. The salamander does not hibernate completely, but will move about in the winter. Congregations can be observed in captivity. These can be produced by insufficient moisture, cold or a lack of suitable cover.

The breaking off of tails has often been reported, and the method described. However, it is not necessarily of frequent occurrence. I have collected over a hundred red-backs without the loss of a tail, although usually one animal in twenty-five will break its tail. The action is worthy of further study, because at times tails are broken after only a slight stimulus, while at other times the tail remains intact after the roughest sort of treatment to the animal. It has been noticed that with animals freshly killed in Bouin's fixing fluid, the tail may fall off with gentle handling, although the skin had not been broken before the handling.

Both Jordan and Kingsley (cited from Cochran, 1911, p. 337) state that *P. cinereus* is nocturnal. While this is true, it is certain also that it feeds during the day in the darkness under stones. One finds individuals, for example, with worms dangling from their mouths, invading ant colonies, etc.

On the whole *P. cinereus* is a timid animal, exhibiting none of the pugnacity of Desmognathus or Pseudotriton.[2] In captivity, I have often seen Plethodon sent scurrying when nipped by the mandibles of a small ground beetle.

[2] I have seen *P. ruber* seize by the neck an attacking garter snake one and one-half feet in length, and then hold on tightly while I picked the snake from the ground. After releasing the snake, the salamander then snapped at my finger.

BREEDING

From observations on general body size and also on the conditions of the gonad, which has been studied microscopically as well as macroscopically, I agree with Blanchard that the males mature in the second year, and deposit their spermatophores a few months after their second birthday. The females mature the following spring. Eggs were laid in 1934 about the middle of June, which is the time Piersol (1910) has given as the earliest date at Toronto. The clutches I found were made up of from three to twelve eggs. Hatching occurred about August 5, which is a month earlier than Piersol reported. The males deposited their spermatophores about the last week in October.

The sex ratio of the adults is 1:1. I have found no correlation between sex and color phase. Furthermore, there is no evidence of partial hermaphroditism, such as the presence of large oviducts in the male, etc.

Maternal solicitude is usually, although not always, in evidence. If a female coiled about her eggs is disturbed, she will move slowly away until she finds a concealed spot from which she will then furtively watch the clutch. But females will leave the eggs in order to find food. Also, one female was found with three eggs of the species contained in her stomach. Piersol (1914) reported that a female kept in a terrarium once swallowed two eggs, and later regurgitated them.

The larvae remain with the female for from three to four weeks after hatching and then gradually disperse. At the time of hatching there is still yolk present and the intestine is incompletely formed. The larvae do not begin to feed until about the third week. The first food consists of small insects.

As Piersol (1910) pointed out, the growth rate varies widely. During the early weeks after hatching, growth is slow, but two months after hatching the larvae may range from 16 to 24 mm in body length. Some larvae

continue to grow slowly, for the following spring (May) very small individuals can be found.

Montgomery (1901) has given a brief description of the embryology of *P. cinereus,* based on a study of five eggs from the same clutch, and Piersol (1910) has recorded some of the events in development. My material does not constitute a developmental series worthy of detailed study, but several gross features of larval development may be recorded.

Piersol has suggested that the pronounced development of the hind limbs of the embryo may be attributed to the leaping peculiarity of the red-backed salamander. However, both Eurycea and Desmognathus larvae have stout hind limbs, and the adult Plethodontid salamanders in general have heavier hind limbs than fore limbs. So, it is perhaps better to correlate the pronounced development of hind limbs in the embryo with general locomotion, rather than with the leaping ability.

The larvae shed their skins soon after hatching. In specimens preserved just before the time of hatching, the epidermis is loose. A week after hatching, however, all signs of a loose epidermis have disappeared.

Belly pigmentation requires about three weeks after hatching to develop fully. Prior to this time the belly is white or faintly speckled. During the development of the black phase, the dorsal pattern underlying the black color is lighter and more in evidence. However, it is irregular and can easily be distinguished from the red phase pattern.

So far as I am aware, Piersol has not published a detailed account of the embryology of *P. cinereus,* as promised in his 1910 paper. It is highly desirable that the embryology of this animal be studied, particularly in regard to the expression and suppression of aquatic larval characters. Complete adaptation to a terrestrial mode of life is one of the most interesting aspects of the evolution of modern amphibia. Moreover, among the Plethodontidae one finds that the species show a grada-

tion from those with a pronounced aquatic dependency to those that are completely terrestrial. A study of the embryology of these varied forms from the comparative standpoint should reveal particularly valuable information.

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THE MECHANISM OF BUD VARIATION

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DARWIN (1882) first called general attention to the significance of bud variation. Since then many observations have been recorded in the literature of the subject (*cf.* Cramer, 1907). Bud variation frequently plays an important rôle in practical science. One of the most laborious studies in this field was made with *Citrus,* particularly the data collected by Shamel and his collaborators in America, and by Tanaka in Japan (*cf.* Tanaka, 1932).

The mechanism of bud variation is based on Baur's theory of periclinal chimeras (1909), which was supported and extended by later investigators, especially Krumbholz (1925), Chittenden (1927), Lange (1927), Massey (1928), Imai (1931) and others. Noack (1922) and others, however, opposed it, suggesting in its place the theory of somatic segregation, but this view runs counter to the general genetic evidences. Recently, the idea of mutable genes has aided greatly in a clear understanding of the mechanism of bud variation (Imai, 1934).

POSSIBLE TYPES OF PERICLINALS

The possible types of periclinal chimeras depend on the number of histogens from which the plant body is developed. Most dicotyledons are trihistogenic, while others, and probably all monocotyledons, are composed of two layers of histogens (*cf.* Imai, 1934, in press, b). Much of the obscurity in connection with the various types of periclinals has been cleared up in the case of the trihistogenic plant, the Japanese morning glory, a description of which will be made here mainly in regard to its bearing on mutable genes. In this plant, three layers of histogens develop the ectohistogen into epidermis of stems and leaves, the mesohistogen into the outer and

marginal mesophyll of leaves and the outer cortex of stems, and the endohistogen into their innermost tissues. This is also the case with other general trihistogenic dicotyledons, although there are a few exceptions (Imai, in press, b). The six possible types of periclinals, as shown in Fig. 1, are either mono- or diheterogeneous for

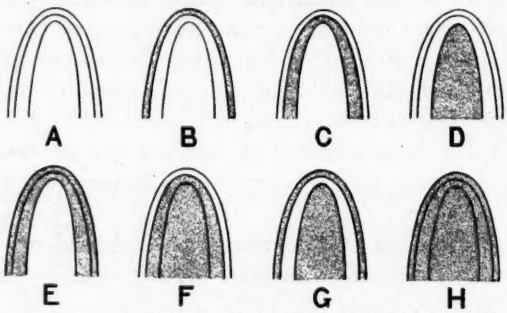


FIG. 1. Diagram showing mutated homo- and heterogeneous growing points (B–H) in comparison with the original (A). The white parts are the original unchanged histogens and the dotted parts the mutated histogens.

tissues having a mutant character, the types being as follows:

- Heterogeneous
  - Monoheterogeneous
    1. Ectohistogenic (Fig. 1B)
    2. Mesohistogenic (Fig. 1C)
    3. Endohistogenic (Fig. 1D)
  - Diheterogeneous
    - Duplicative
      4. Ecto-mesohistogenic (Fig. 1E)
      5. Meso-endohistogenic (Fig. 1F)
    - Alternative
      6. Ecto-endohistogenic (Fig. 1G)
- Homogeneous (Fig. 1H)

Of compound periclinals, the ecto-endoheterogeneous type is the most complicated, the arrangement of the different tissues being alternative, while the others are duplicative.

THE STRUCTURE OF BUD VARIATION

Plants in which mutable genes operate frequently exhibit bud variation due to changes in genes in somatogenesis. Three monoheterogeneous periclinals have been identified in the flecked stock (Fig. 2A) of *Pharbitis* by Imai (1931, 1934). Kihara (1934) also obtained similar

results from the same character. Gene flecked being mutable, it gives anthocyanin flecks to the otherwise white corollas, resulting sometimes in homo- or hetero-

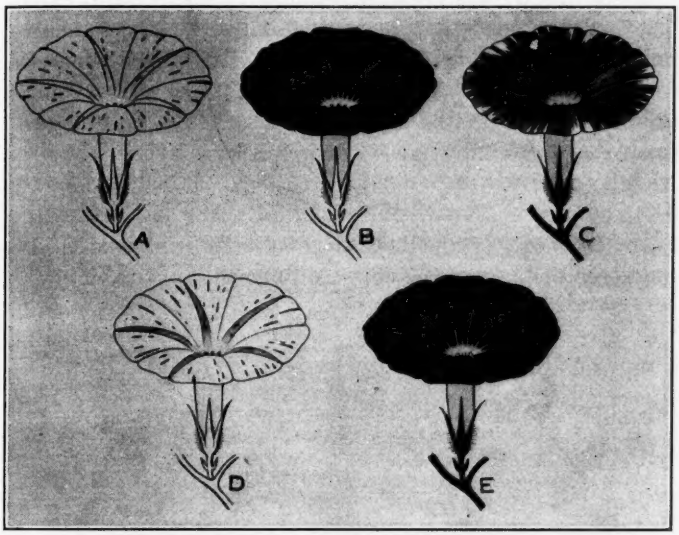


FIG. 2. Flecked prototype (A) of *Pharbitis*, and its monoheterogeneous (B, C, D) and homogeneous (E) sports.

geneous bud variations with the following characteristics:

(1) Ectoheterogeneous (Fig. 2B): Flower self-colored; stem green.

(2) Mesoheterogeneous (Fig. 2C): Flower lightly colored, with white fringing and dark flecks; stem colored by anthocyanin.

(3) Endoheterogeneous (Fig. 2D): Flower flecked, with tinged rays; stem green.

Anatomical observations show that anthocyanin pigment in various degrees is contained in all the tissues of the corollas, being most intense in the epidermis and less so in the inner tissues, while only the subepidermal cells are colored in the stems. Therefore, ectoheterogeneous periclinals, in which the ectohistogen carries the mutated self-colored gene (normal allele to flecked), should bear self-colored flowers on green stems, and mesoheterogene-

ous periclinals, in which the mesohistogen is mutated, should have colored stems and bear lightly colored flowers with white fringings and dark flecks. The light color of the flowers results from pigments contained in the inner tissue (mesohistogen) underlying the epidermis, the white fringing from the massive development of the colorless ectohistogen, and the flecks from the somatic mutation that occurs in the ectohistogen. Endoheterogeneous periclinals, however, should have green stems on which should bloom flecked flowers with tinged rays, since the rays are believed to consist of three histogens, the intra-rays of the corollas, except the fringed parts, having developed from the ecto- and mesohistogens (Fig. 3).

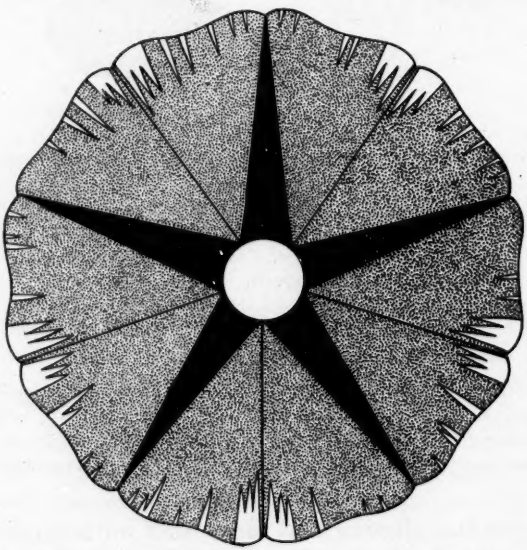


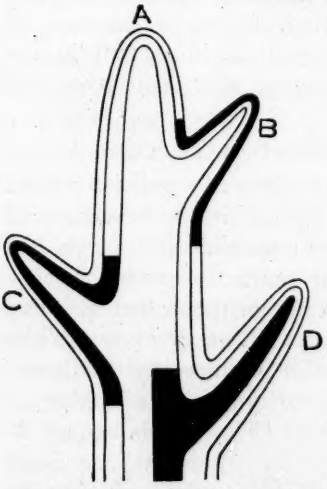
FIG. 3. Flower of *Pharbitis*, showing the number of histogens from which the respective parts are developed; namely, the white parts from one histogen, the dotted from two, and the black from three histogens.

Breeding experiments fully bear out this view, in that the ecto- and endoheterogeneous sports bred to flecked, whereas the mesoheterogeneous sports generally segregated, monogenically, self-colored and flecked. The

segregation is due to the heterozygous constitution of the mesohistogen, induced by gene mutation from flecked to self-colored.

The diheterogeneous periclinals in the flecked are difficult to distinguish from certain monoheterogeneous and homogeneous sports, namely, the ecto-mesoheterogeneous from the homogeneous, the meso-endoheterogeneous from the mesoheterogeneous, and the ecto-endoheterogeneous from the ectoheterogeneous, although these diheterogeneous cases probably occur but very rarely. Homogeneous bud variation, which bears self-colored flowers and stems (Fig. 2E), however, arises rather frequently in the flecked.

Bud variation occurs when the mutated cell propagates to cover a growing point (Fig. 4). The sports as a rule should therefore be monoheterogeneous, since bud varia-



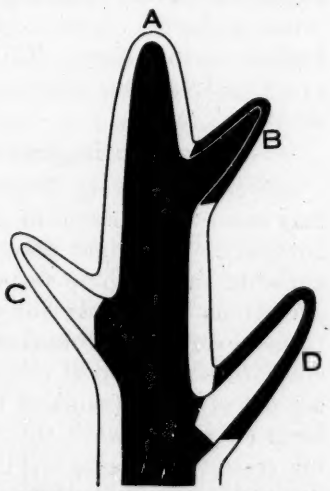


FIG. 4 (left). Origin of the three monoheterogeneous bud variations (B, C, D) on the original form (A). Black parts indicate mutant histogens.

FIG. 5 (right). Diagram showing bud variation due to somatic rearrangement of tissues. A, the original white-over-green periclinal, regardless of ectohistogen, which last is omitted from the drawing. B, C, D are derivative types; green, white, and reversal respectively. Black and white parts represent green and white histogens, respectively.

tion may be induced by single mutation, although plants that have germinated direct from seeds may also bear homogeneous bud variation. Embryonic development, however, differs entirely from later somatogenesis, the triplicately arranged histogens being at first formed in the plumules of the embryos with no differentiation yet in general embryonic tissues, so that generally speaking, it is only when the mutated cells contribute all the cells for the formation of the plumules that homogeneous bud variation could result, unless further complications occur. Therefore, should mutation manifest any characteristics in the plant, the presence of homogeneous sports can be traced to the base of the stem near the ground. In the flecked *Pharbitis,* this is easily done by following the anthocyanin stripes running down the stems. On trees and shrubs, excepting gymnosperms, the occurrence of homogeneous bud variation regardless of embryonic mutation is hardly to be expected, except in the case of further complications. This point is important in the practical treatment of sports, especially fruit trees and shrubs.

Somatic Rearrangement of Tissues

Although not very frequent, somatic rearrangement may occur as the result of irregularities in the course of ontogeny. Periclinal plants in general put forth bud variation due to these somatic rearrangements. White-over-green periclinals, for example, throw out reversal (green-over-white) branches as well as green and white ones. The green sport (Fig. 5B) is caused by the throwing out of white (skin) of the original white-over-green form (Fig. 5A), while the white (Fig. 5C) is caused by the green (core) being left out. In either case, one tissue develops into two (or three) histogens by replacing the other tissues. In the reversal sport (Fig. 5D), the arrangement is quite reversed. Although the cause of somatic rearrangement is not very clear, unequal activities of the different histogens in the development of the primordial buds may be an inducing cause. If the green

endohistogen should begin to develop earlier or more vigorously than the white mesohistogen in the white-over-green periclinal, we may have a green sport as the result of the white tissue being thrown out. The reverse will induce the production of a white sport. In some cases there is a marked difference in the frequency with which the two homogeneous sports are produced on the white-over-green periclinals, which clearly seems to depend on the great inequality in the developing power of the two histogens. The *modus operandi* of the reversal arrangement is a little more complicated. If we suppose a case in which the green endohistogen began development a little earlier than the white mesohistogen, then the mutual powers for the development of a bud possessed by the two histogens may help to bring about the reversal arrangement.

Since bud variation should as a rule be monoheterogeneous, secondary changes due to somatic rearrangement may occur. Beside chlorophyll chimeras, some horticultural strains having periclinal constitutions are found (Bateson, 1916, 1921; Imai, in press, a). These forms probably arose through bud variation. In fact, they give at times secondary sports induced by somatic rearrangement of tissues.

Adventitious buds from the roots of endoheterogeneous periclinals result in bud variation. The buds in this case being endogeneous, the ectohistogen develops into three histogens of new shoots, so that in such periclinals, root-cutting induces bud variation (Bateson, 1916, 1921; Chittenden, 1927).

Simpler Cases

As fully discussed elsewhere (Imai, 1934, in press, b), some dicotyledons, and probably all monocotyledons, consist of two layers of histogens, in which cases the periclinal structure is simpler than in those of trihistogenic plants. Bud variation due to mutation therefore should be either monoheterogeneous or homogeneous in dihistogenic plants that germinate directly from seeds, while in

trees and shrubs, in which the change generally occurs regardless of embryonic mutation, it may be monoheterogeneous in general.

From his anatomical studies, Douliot (1890) concluded that the plant body of gymnosperms is developed from a single initial cell at a growing point, while Koch (1891) insisted on its multicellular origin. Douliot also believed that each histogen of an angiosperm consists of an initial cell, which, however, is an idea that does not reconcile with our present general knowledge of multicellular constitution. Seeing that bud variation at times shows a sectorial chimera, even in gymnosperms, the multicellular view must be correct. So far as I am aware, no cases of periclinals have been detected in gymnosperms. This plant group is supposed to be developed from a single histogen, which consists of only two or three initial cells. Since in gymnosperms, therefore, bud variation is homogeneous, it should always be propagated as a constant form by cutting or grafting or even by root-cutting. This is a characteristic of sports in this plant group.

Descending a step lower in the plant group, the stem or frond of a fern arises from an apical cell, the origination being unicellular. Bud variation in ferns and other lower plants is quite simple, neither periclinal nor typical sectorials, but only homogeneous ones, being obtainable.

CONCLUSION

The possible types of bud variation depend on the number of histogenic layers from which the plant body is developed. Generally, dicotyledons are trihistogenic, whereas some dicotyledons and probably all monocotyledons are dihistogenic. Gymnosperms, however, have single histogens. There are six possible types of periclinals in trihistogenic plants, including three monoheterogeneous, two duplicative and one alternative diheterogeneous. Bud variation is generally monoheterogeneous, although embryonic mutation results in homogeneous sports as well. In dihistogenic plants, however, bud variation may be either monoheterogeneous or

homogeneous, while in gymnosperms only homogeneous sports are obtainable, except sectorials.

Somatic rearrangement of tissues may induce bud variation secondarily in the periclinal stocks. The new strains that have appeared by bud variation may constantly, though not frequently, bear such sports—a point that can not be overlooked or ignored in practice. Since in gymnosperms and ferns, however, the sports are homogeneous, this point may be disregarded so far as their vegetative propagation is concerned.

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BEHAVIOR OF MIXED POPULATIONS AND THE PROBLEM OF NATURAL SELECTION

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THE splendid development of genetic theories of natural selection, dealing with the problem on the exact quantitative basis, is well known. Starting with the consideration of two types or species which do not cross each other and making certain simple assumptions, they enable us to calculate the rate at which the better fitted type is displacing (and will expel to the very last) the less fitted one. But when we approach the conditions of competition in nature, take into account the ecological situation, make certain experimental investigations and write the equation of interaction between the competing species in a more general form, the complexity of the problem under consideration is obvious. It is hardly to be questioned, therefore, that the problem of the growth of a mixed population of two species is worthy of a very careful study so that it could give a sound basis for the development of complicated genetic theories of natural selection.

In the last two years the growth of species competing or helping each other was discussed by several authors. We can mention a book by Lotka (1934) and Kostitzin (1934), a paper by Winsor (1934), and two books by Gause (1934, 1935). The present paper gives an account of our recent theoretical investigations of the problem and summarizes the whole theory of growth of mixed populations, pointing out those conclusions that have already been confirmed by experimental investigations of one of the authors.

(1) Equation of Interaction Between Species

The interaction between two competing species was expressed by Volterra (1926), Lotka (1932) and Gause (1932) in the form of a differential equation:

$$\begin{aligned} dN_1/dt &= b_1N_1 \frac{K_1 - N_1 - \alpha N_2}{K_1} \\ dN_2/dt &= b_2N_2 \frac{K_2 - N_2 - \beta N_1}{K_2} \end{aligned} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

It was discussed in detail by Gause (1934), and there is no need to repeat it here. Let us only note that (1) this expression is generally true only for very simple populations of yeast cells, (2) the coefficients α and β often change in the course of the growth of the mixed population, and (3) according to Gause (1935) this equation has certain serious limitations. It is supposed in it that in the process of displacing one species by another from the saturating population the properties of these species (coefficients b, α, β) are practically the same as in the initial stages of growth taking place in almost unoccupied microcosm. In biological associations, however, this condition is often unfulfilled, as the properties of individuals of a certain species undergo considerable alterations under the influence of crowding. To avoid this difficulty in experimental investigations, it is sometimes convenient to perform regular and small dilutions of saturating population, keeping it continuously in the process of active growth (such variations in density often take place in field conditions):

$$\begin{aligned} dN_1/dt &= b_1N_1 \frac{K_1 - N_1 - \alpha N_2}{K_1} - mN_1 \\ dN_2/dt &= b_2N_2 \frac{K_2 - N_2 - \beta N_1}{K_2} - mN_2 \end{aligned} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where $m < b$.

(2) Strong Mutual Depression of Species

An analysis of the properties of the equations (1) and (2) gives us the possibility of discussing certain essential types of the struggle for existence between species.

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(2) Strong Mutual Depression of Species

An analysis of the properties of the equations (1) and (2) gives us the possibility of discussing certain essential types of the struggle for existence between species.

The consideration of properties of "singular points" and of the movement of integral curves between these points shows that when $\alpha > K_1/K_2$ and $\beta > K_2/K_1$ (equation 1) or $\alpha/1-\frac{m}{b_1} > \frac{K_1}{K_2}/1-\frac{m}{b_2}$ and $\beta/1-\frac{m}{b_2} > \frac{K_2}{K_1}/1-\frac{m}{b_1}$ (equation 2) either the first species will entirely displace the second, or the second entirely displace the first, depending on numerical relations between species at the beginning of their competition (Fig. 1). The biological

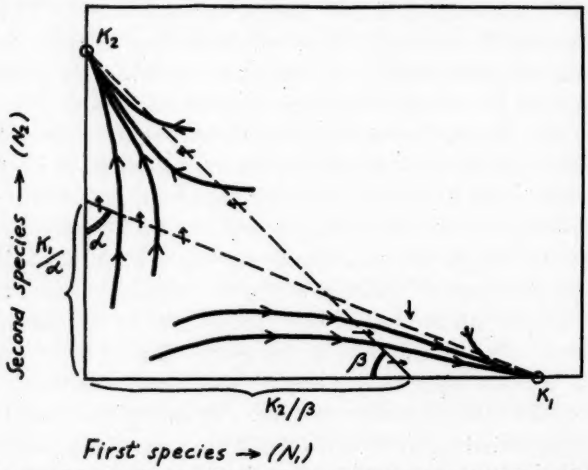

FIG. 1. Curves of interaction in the case of strong mutual depression of species. The coefficients α and β are the tangents of the corresponding angles.

meaning of this conclusion consists in the fact that if the mutual depression of two species is strong the *less numerous* species (whose concentration lies below a certain separatriss) will always disappear in the population. This conclusion has not yet been confirmed by direct experimental methods.

(3) COMPETITION BETWEEN TWO SPECIES BELONGING TO THE SAME ECOLOGICAL NICHE

Let us admit that two species consume one and the same single foodstuff or, if they consume a mixed diet,

that the proportion of each ingredient of the diet which they consume is the same for both species. Then if an individual of second species consumes per unit of time twice as much food as the first, it will influence the unutilized opportunity for the growth of the first species twice more than the first species decreases in its growth (α will be equal to 2). But the reverse action of the first species upon the second will be here twice feebler than the action of the second species upon itself ($\beta = 1/2$). Under such circumstances the following condition could be fulfilled: $\alpha < K_1/K_2$ and $\beta > K_2/K_1$ (equation 1) or $\alpha/1 - \frac{m}{b_1} < \frac{K_1}{K_2}/1 - \frac{m}{b_2}$ and $\beta/1 - \frac{m}{b_2} > \frac{K_2}{K_1}/1 - \frac{m}{b_1}$ (equation 2) and, theoretically, only N_1 survives in the mixed population independently of initial concentrations of the species (Fig. 2). When $\alpha > K_1/K_2$ and $\beta < K_2/K_1$ survive N_2,

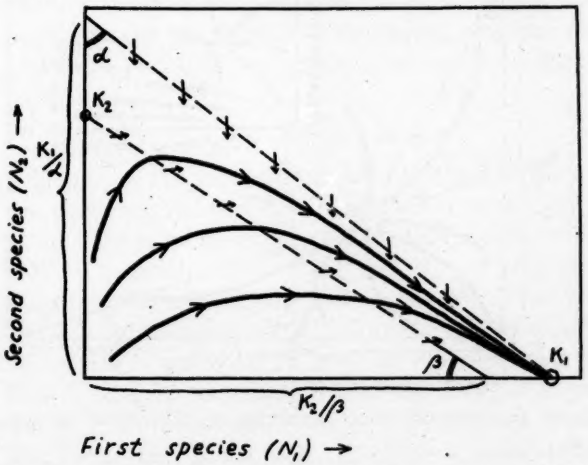

FIG. 2. Curves of interaction in the case of competition between two species belonging to the same ecological niche.

or always the "better adapted" species. A similar conclusion was reached by Haldane (1924), Volterra (1926) and Lotka (1932), and an experimental demonstration of the entire displacing of one species by another in a mixed population of this kind was given by Gause (1934, 1935).

(4) SLIGHT MUTUAL DEPRESSION OF SPECIES

When two species belong to different ecological niches in the microcosm and, for instance, in addition to common food for each of the species a special kind of food is available that can not be so effectively obtained or consumed by another species, the mutual depression of these species will be less. Here the following condition may be fulfilled: $\alpha < K_1/K_2$ and $\beta < K_2/K_1$ (equation 1), or $\alpha/1-\frac{m}{b_1} < \frac{K_1}{K_2}/1-\frac{m}{b_2}$ and $\beta/1-\frac{m}{b_2} < \frac{K_2}{K_1}/1-\frac{m}{b_1}$ (equation 2). Theoretical analysis shows that in this case the competition leads to a certain stable population consisting of both species instead of the entire displacement of one of them by another (Fig. 3). An important feature here

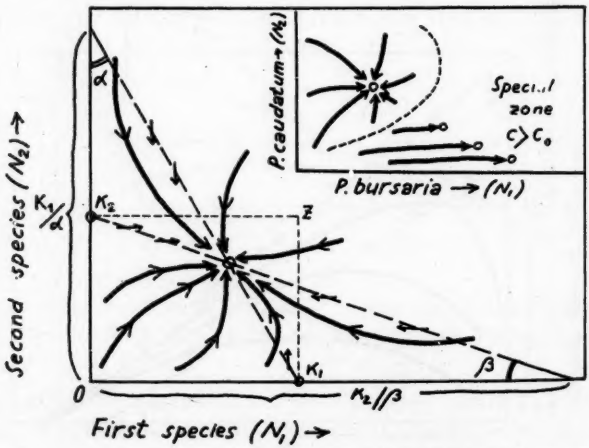

FIG. 3. Curves of interaction in the case of slight mutual depression of species.

also is the "regulation" of composition of the stable population: the disturbance of the stable combination of species leads automatically to the reestablishment of stable combination, in which each of the species is driven into its "niche." Theoretical possibility of an infinite survival of both species in a mixed population (or of a

"knot" on the surface N_1N_2) was mentioned by Lotka (1932) and more definitely by Winsor (1934), who put the condition for it in the form: $\alpha\beta < 1$. However, this condition shows only that *if a "singular point" exists* it will be a "knot" (but there also can be no singular point when $\alpha\beta < 1$). Our conditions $\alpha < K_1/K_2$ and $\beta < K_2/K_1$ guarantee both the existence of the singular point and the fact that it is a "knot." For instance, if $\alpha = 5{,}5$; $\beta = 0{,}12$; $K_1 = 75$; $K_2 = 15$, then $\alpha\beta = 0{,}66$ and is < 1, but there is no singular point in the center of the map of integral curves because $5{,}5 > 5$ $(K_1/K_2 = 5)$ and $0{,}12 < 0{,}2$ $(K_2/K_1 = 0{,}2)$.

An experimental demonstration of the existence of a stable mixed population of two species was given by Gause (1935) in the case of two protozoa, one of which (*Paramecium caudatum* or *P. aurelia*) consumes more effectively bacterial components of a mixed diet suspended in the upper layer of the liquid, whereas the other (*P. bursaria*) prefers yeast cells sedimenting on the bottom.

(5) Special Zones

Experiments show that a definite type of the struggle for existence is often observed only in a certain part of the map of integral curves or, in other words, only under certain concentrations of N_1 and N_2. For instance, in the case of *P. caudatum* and *P. bursaria* (Gause, 1935) the reestablishment of a disturbed stable combination of species is not possible from *all* the points on the surface N_1N_2. If we make the concentration of *P. bursaria* too high, *P. caudatum* is not in a position to drive it out into its former place, and we obtain a stable population with the decreased concentration of *P. caudatum* (Fig. 3, above). In short, when *P. caudatum* enters a young biocoenose with the low concentration of *P. bursaria* the situation for its development appears to be much more favorable than in the case of an older biocoenose. Experiments have shown that this is due to the fact that

P. caudatum is much more sensitive than *P. bursaria* to the waste products accumulating in the daily renewed medium.

Let us consider briefly how the equation of the struggle for existence changes in this particular case. We know (Gause, 1935) that the coefficient of multiplication of *P. caudatum* (b_2) is inhibited by waste products of N_1 and N_2 (let us denote them C), and the expression of the biotic potential will be: $b_2 f(C) N_2$. The accumulation of C will be given by the difference of two factors, one of which depends on their production by N_1 and N_2, and another which represents their removal by washing the cultures in the experiment:

$$dN_1/dt = b_1 N_1 \frac{K_1 - N_1 - \alpha N_2}{K_1}$$

$$dN_2/dt = b_2 f(C) N_2 \frac{K_2 - N_2 - \beta N_1}{K_2}$$

$$dC/dt = f(N_1 N_2) - nC$$

Here $f(N_1 N_2)$ depends more on N_1 than on N_2. When the concentration of C rises above a certain threshold value C_0 the value $b_2 f(C)$ becomes zero and *P. caudatum* (N_2) ceases growing. The growth of N_1 continues until $K_1 - N_1 - \alpha N_2 = 0$. By introducing *P. caudatum* into even more and more dense cultures of *P. bursaria* we obtain a series of stable populations with even lower and lower levels of *P. caudatum*. But when the concentration of C is below C_0 the interaction between the species leads to the "classical" stable population. The general appearance of the curves is shown on Fig. 3, above.

It is to be remarked that we have here three equations with three unknowns, and therefore the integral curves ought to be reproduced in space, instead of surface. However, the structure of our equations enables us to represent their solutions in the region of singular points on surface $N_1 N_2$. Indeed, the stationary states are given by the equations: $dN_1/dt = 0$, $dN_2/dt = 0$, $dC/dt = 0$. The last expression gives C if N_1 and N_2 are known, and the first two N_1 and N_2 independently of C (when $C < C_0$).

If $C > C_0$ the growth of N_2 stops and N_1 is obtained independently of C.

In our real experiments the situation is somewhat more complicated in connection with diurnal rhythm of accumulation of waste products and dilutions of both species. We attempt here to give only a general idea of separate zones introducing new complicating circumstances and changing differential equations of competition.

(6) Symbiosis

Certain symbiotic equations have recently been considered by Kostitzin (1934). Let us analyze here briefly the properties of the basic equation (1) in the case of mutual aid of two species to each other:

$$\begin{aligned} dN_1/dt &= b_1N_1 \frac{K_1 - N_1 + \alpha N_2}{K_1} \\ dN_2/dt &= b_2N_2 \frac{K_2 - N_2 + \beta N_1}{K_2} \end{aligned} \qquad \text{(3)}$$

Three following stationary states ought to be mentioned: (1) $N_1 = 0$, $N_2 = 0$; (2) $N_1 = 0$, $N_2 = K_2$; (3) $N_2 = 0$, $N_1 = K_1$. The fourth $N_1 = K_1 + \alpha K_2/1 - \alpha\beta$, $N_2 = K_2 + \beta K_1/1 - \alpha\beta$ will exist only when $\alpha\beta < 1$. Investigation shows that the properties of the singular points are as follows: (1) "unstable knot," (2) "saddle," (3) "saddle" and (4) "stable knot" when $1 - \alpha\beta > 0$. Fig. 4 (above) gives the curves of interaction between species for the case of symbiosis. We have here a certain natural extension of the principle included in Fig. 3. There, with the slight mutual depression of species, the position of the stable "knot" corresponding to a stationary population was determined by intersection of isoclines of horizontal with that of vertical tangents which took place within the tetragon OK_2ZK_1. In the case of mutual aid this intersection lies outside of tetragon and in the mixed population both species together attain larger biomasses than separately. As coefficients of mutual aid α and β increase the "knot" continuously moves up and right-

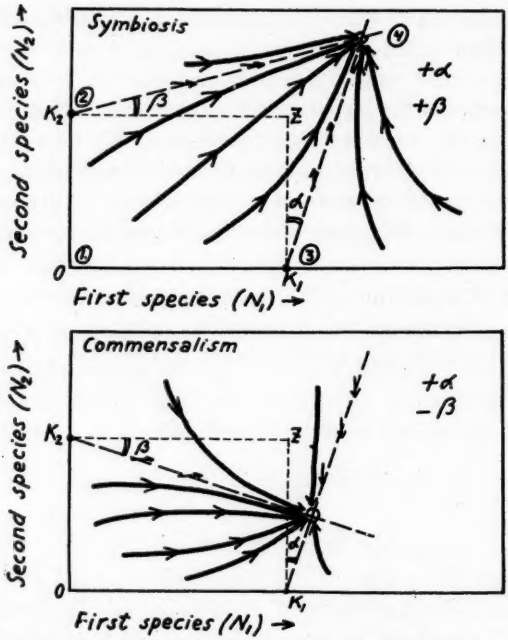

FIG. 4. Curves of interaction between species in the case of symbiosis and of commensalism.

wards and finally with $\alpha\beta = 1$ passes into infinity. It is evident that in a limited microcosm we can not expect infinite populations, and certain observations (Gause, 1934) indicate that the coefficient of mutual aid can considerably decrease in the course of growth of the culture. The case of symbiosis awaits its exact experimental investigation (the experiments of Gause referred to deal with one-sided symbiosis or commensalism and are not complete). Recent studies on artificial symbiosis (Buchsbaum, 1934) open here a new way for attack.

(7) COMMENSALISM

When the first species has a gain due to the presence of the second ($+ \alpha N_2$) and in its turn slightly depresses the second ($-\beta N_1$) we have, speaking biologically, com-

mensalism. The corresponding curves of interaction are reproduced on Fig. 4, below. Here the first species in a mixed population attains larger, and the second lower level than separately. As the degree of depression (β) increases the "knot" corresponding to the stable population gradually sinks, and with it the biomass of both species. Finally, the isocline of horizontal tangents crosses K_1 and subsequently remains the pure population of the first species only ($K_2 < K_1\beta$). In this way the system of equations of interaction gives a definite idea of the continuous passage from mutual depression to commensalism and symbiosis of species which is sometimes observed in biological investigations (Yonge, 1934). It is to be hoped that the accumulation of the experimental material will give us the possibility of adopting these equations as the basis of classification of the corresponding biological systems.

(8) The Action of External Factor on Mixed Population

Let us assume that the process of interaction between two competing species (equation 2) depends on a certain external factor, for instance, on temperature, attaining these or those fixed values. With the alteration of temperature the curves of the struggle for existence will continuously change. But at certain special or *bifurcation* values of temperature they will change *qualitatively;* in other terms, will change their topological structure—character and number of basic elements or "singular points." The *change of stability* of the system will take place. At temperatures *below* the bifurcation we can observe one type of the struggle for existence (for instance, strong mutual depression) with the typical stable pure population, and *above* the bifurcation slight mutual depression with the corresponding stable mixed population.

Let us analyze the case of the system (2) where the

conditions of the four types of the struggle for existence will be:

$$1^\circ\ \alpha/1-\frac{m}{b_1}>\frac{K_1}{K_2}/1-\frac{m}{b_2}\ \text{and}\ \beta/1-\frac{m}{b_2}>\frac{K_2}{K_1}/1-\frac{m}{b_1}\ \text{(strong depression)}$$
$$2^\circ\ \text{“}\ <\ \text{“}\ \text{and}\ \text{“}\ >\ \text{“}\ \text{(remains } N_1\text{)}$$
$$3^\circ\ \text{“}\ >\ \text{“}\ \text{and}\ \text{“}\ <\ \text{“}\ \text{(remains } N_2\text{)}$$
$$4^\circ\ \text{“}\ <\ \text{“}\ \text{and}\ \text{“}\ <\ \text{“}\ \text{(slight depression)}$$

We can admit that with the increase of temperature the coefficient of multiplication (b) in each species increases, but in the first species more rapidly. To simplify the calculations let us express this relation by a straight line, although every exponential relation will not change the essence of our conclusions: $b_1 = B_1 + \lambda_1 t$, $b_2 = B_2 + \lambda_2 t$, where $\lambda_2 < \lambda_1$ and $B_1 = B_2 = 0$. Since the calculation represents the very first approximation to the actual state of affairs, we neglect the action of temperature upon all other parameters. We assume also the dilution (m) less than the multiplication (b), so that $1-\frac{m}{b_1}>0$ and $1-\frac{m}{b_2}>0$. To separate variables from constant values we put $1-\frac{m}{b_2}/1-\frac{m}{b_1}=F(t)$, where t is temperature, and obtain the conditions of the four types of the competition:

$$1^\circ\ F > K_1/K_2\,\alpha\ \text{and}\ F > \beta\,K_1/K_2$$
$$2^\circ\ \text{“} < \text{“}\ \text{and}\ \text{“} > \text{“}$$
$$3^\circ\ \text{“} > \text{“}\ \text{and}\ \text{“} < \text{“}$$
$$4^\circ\ \text{“} < \text{“}\ \text{and}\ \text{“} < \text{“}$$

Denoting $F(t)=y$ and analyzing the curve of this function we can conclude that as y passes certain critical values, y_1, y_2 . . . etc., defined by non-equalities written above, the type of the stable system undergoes a change. Since $y=t-\frac{m}{\lambda_2}/t-\frac{m}{\lambda_1}$ we can easily calculate a simple example of temperature bifurcation (Fig. 5), which shows how one type of competition is transformed into another.

Summarizing the essence of theoretical calculations we

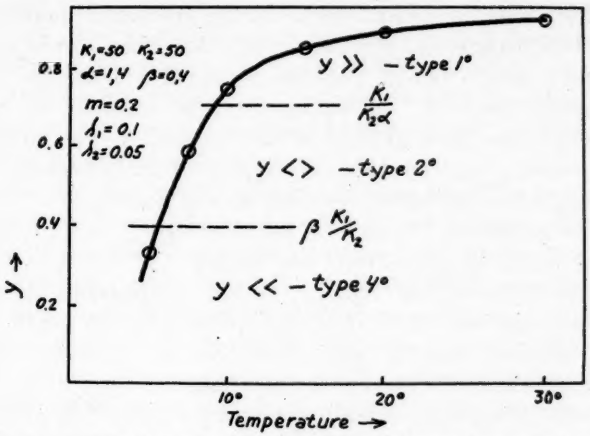

FIG. 5. A theoretical example of the action of temperature upon a mixed population of two species.

note that according to our admissions both species can separately dwell in the habitat and in both the rate of multiplication increases with temperature, but only in a different degree. As a result above a certain threshold one of them can not withstand competition, and bitypic system (4°) is transformed into monotypic (2°). This takes place with the fixed value of dilution and is due to purely biocoenotic (competition) and not physiological (temperature threshold) causes. From the above the conclusion may be drawn that *under the influence of temperature* (and of any other) *gradient a mixed population separates into a number of distinct types.* This conclusion can be confirmed experimentally (Gause, in press) and it is to be remarked that under the complicated conditions of actual habitats we often meet an interplay of biocoenotic and physiological causes of bifurcation. The history of previous temperature changes in the system may also be very important.

(9) Rate of Natural Selection

The problem of the rate of natural selection in a population of two species may be reduced to the calculation

of the "rate of stabilization" of the system of equations of the struggle for existence (1). In other words, how rapidly is the mixed population purified from the less adapted species? Unfortunately, the solution of this problem in a general form is difficult, and we can analyze here only a special case of slight mutual depression of two species leading to a stable mixed population.

The equation (1) can be written: $dx/dt = Ax\,(1 - ax + by)$ and $dy/dt = By\,(1 - cy + dx)$. If $b = d = 0$ we obtain usual logistic curves for the independent growth of the species. Now if b and $d \neq 0$ and are small the solutions of logistic equations can be expanded into series and the orders of b and d higher than first neglected. We obtain certain corrections inhibiting growth (since b and d are negative): $x = x_0 - x_1$. If $A \neq B$ the solutions can be obtained in the form of integrals which can not be taken in simple functions, but if $A = B$ we obtain:

$$x = x_0 - x_1 = \frac{\bar{x}_0}{a\bar{x}_0 + (1 - a\bar{x}_0)e^{-At}} - \frac{b}{ac} KK_1 \frac{e^{-At}}{(e^{-At} + K)^2}\left[\frac{K - K_1}{K^2} \lg \frac{e^{-At} + K_1}{e^{-At}(1 + K_1)} - \frac{K}{K_1}(e^{At} - 1)\right]$$

and an analogous expression for y, where $K = x_0 a/1 - a\bar{x}_0$, $K_1 = \bar{y}_0 c/1 - c\bar{y}_0$, and $\bar{x}_0 = x_{t=0}$, $\bar{y}_0 = y_{t=0}$.

From the view-point of the theory of natural selection it would be particularly interesting to obtain a solution for the case of the strong competition, which would help to connect the method of Volterra (1926) with that of Haldane (1924) and Ludwig (1933). It is to be hoped that further analysis will complete this investigation.

(10) CONCLUSIONS

The theory of the growth of mixed population of two species is already confirmed in many points by direct experimental investigations, and it can be discussed from two different angles. Firstly, it is directly connected with the problem of natural selection. We distinctly see here how the evolutionary process of expelling the less

adapted species by a better adapted one changes with the stage of growth of the population, the relation in concentrations of the competing types ("special zones") and their "ecological niches." In other words, this theory demonstrates that the relative adaptability of two types is a rather variable feature.

Secondly, the results of our analysis ought to be connected with the problems of modern biocoenology. Regulation of the stable combination of species (a system with the slight mutual depression, commensalism and symbiosis) and the separation of the mixed population into a number of distinct types under the action of temperature (and of any other) gradients sheds a new light on the problem of organization of a biological unit—biocoenose.

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SHORTER ARTICLES AND DISCUSSION

THE CALCULATION OF RELATIVE GROWTH CONSTANTS

Introduction

In the application of the relative growth function, $y = bx^k$, the constants for most of the data have been determined by the graphic method. Since it has been shown that the function is of general applicability to a wide variety of data, it is desirable to make a more precise determination of the constants. The purpose of this paper is to discuss the technique of estimation of the relative growth constants by the method of least squares and to give an adequate and time-saving arrangement of the work on the standard calculating machine.

Derivation of the Constants b and k

The relative growth function in straight form becomes

$$\log y = \log b + k \log x \qquad \text{(I)}$$

The normal equations required to fit a curve of this general type are:

$$\Sigma(\log y) = N \log b + k \Sigma(\log x) \qquad \text{(II)}$$
$$\Sigma(\log x . \log y) = \log b \Sigma(\log x) + k \Sigma(\log^2 x) \qquad \text{(III)}$$

The solution of these simultaneous equations yields the following expression for k:

$$k = \frac{N \Sigma(\log x . \log y) - \Sigma(\log x) \Sigma(\log y)}{N \Sigma(\log^2 x) - [\Sigma(\log x)]^2} \qquad \text{(IV)}$$

One readily recognizes in it the familiar expression of the coefficient of regression of $\log y$ on $\log x$ in terms of raw scores.

Derivation of the Probable Error of k

The probable error of k may be determined in two ways.

A. Since k is essentially a coefficient of regression we may determine the probable error of k from the formula:

$$\text{P.E.}_k = \pm 0.6745 \sqrt{\frac{1 - r^2}{N - 2}} \cdot \frac{\sigma_{\log y}}{\sigma_{\log x}} \qquad \text{(V)}$$

One may rewrite this formula for the raw scores. Expanding it we obtain:

$$\text{P.E.}_k = \pm 0.6745\sqrt{\frac{N\Sigma(\log^2 y) - [\Sigma(\log\ y)]^2}{N\Sigma(\log^2 x) - [\Sigma(\log\ x)]^2} - k^2} \qquad \text{(VI)}$$

B. The probable error of k may also be computed by means of deviations from the regression line, according to the following formula:

$$\text{P.E.}_k = \pm 0.6745\sqrt{\frac{\Sigma(\log\ y - \log\ Y)^2}{(N-2)\ \Sigma(\log\ x - M\log\ x)^2}} \qquad \text{(VII)}$$

where $\log Y = M\log y + k\ (\log x - M\log x)$.

It can be readily shown that the two formulae are identical.

ARRANGEMENT OF WORK ON A STANDARD CALCULATING MACHINE

In order to determine the value of k by method of least squares four summations are needed: $\Sigma(\log x.\log y)$, $\Sigma(\log x)$, $\Sigma(\log y)$ and $\Sigma(\log^2 x)$. They may be obtained in two sets of continuous operations.

To find $\Sigma(log\ x.log\ y)$ *and* $\Sigma\ (log\ y)$*:* Shift the carriage to the extreme left. Lock the extreme unit on the keyboard. Then depress on the extreme right of the keyboard the first value of $\log x$ and multiply it by the corresponding value of $\log y$. Clear the upper dial, *but not the lower one.* Depress the second value of $\log x$ and multiply it by the corresponding second value of $\log y$. Follow this procedure for all values of $\log x$ and $\log y$. Upon completion of this operation, $\Sigma(\log x.\log y)$ is found on the extreme right and the value of $\Sigma(\log y)$ to the left of it. Copy these summations on the work sheet, clear the machine, leaving the extreme left unit locked.

To find $\Sigma(log^2 x)$ *and* $\Sigma(log\ x)$*:* Depress the value of $\log x$, first in the series and multiply it by itself. Repeat the procedure for all the values, clearing only the upper dial. At the end of the operation, $\Sigma(\log^2 x)$ will appear on the right of the lower dial and $\Sigma(\log x)$ to the left, against the locked unit. Copy these summations on the work sheet, unlock the unit and clear the machine.

To find the numerator in (IV)*:* Depress the value $\Sigma(\log x.\log y)$ on the extreme right of the keyboard and multiply it by N. Clear the upper dial and the keyboard. Depress the

value $\Sigma(\log x)$ and multiply it by $\Sigma(\log y)$, *using the minus key.* Copy the result on the work sheet and clear the machine.

To find the denominator in (IV): Depress the value of $\Sigma(\log^2 x)$ and multiply it by N. Clear the upper dial and the keyboard. Depress the value $\Sigma(\log x)$ and multiply it by itself, *using the minus key.* Copy the results on the work sheet and clear the machine.

The value of k is the quotient.

To find the probable error of k: In order to determine the probable error of k two additional summations are needed, namely $\Sigma(\log^2 y)$ and $\Sigma(\log y)$. These summations can be obtained in the way identical with that described for the log x values. Solution of the formula (VI) presents no difficulty. The denominator under the radical is identical with the denominator used in (IV).

DRAWING OF THE LEAST SQUARES LINE

When it is desirable to present the data graphically, the values of x and y are plotted on double logarithmic grid and then two or three points of the least square line are computed analytically to determine the position of this line. This determination of points on the line of least squares may be best arranged by evaluating the transposed regression equation.

$$\log y = M\log y + k \log x - k\, M\log x \qquad \text{(VIII)}$$

The evaluation of this equation for the different values of log x may be done by continuous operation in the following fashion.

Solution of equation (VIII) for the different values of log x: Depress the value $M\log y$ on the keyboard. Shift the carriage from the extreme right to the number of places equal to the number of decimal places in k. (We assume that the number of decimals in log x is the same as in log y). Add $M\log y$ in the machine and then clear the keyboard. Mark off the position of decimals by a pointer. Depress the value of k on the extreme right of the keyboard, shift the carriage to the extreme left and multiply it by the value of $M\log x$, *using the minus key.* The value on the lower dial will be: $M\log y - k\, M\log x$, or the value of log b, the y-intercept.

Without clearing the machine, except for the upper dial, multiply the depressed value of k by the selected values of log x.

On the lower dial the corresponding values of log y will be found. If the values of log y are smaller than the corresponding values of log x, subtract the value Mlog y as the first step and then proceed as before described, using the plus key.

These values of log y may be used for computation of the probable error of k by the formula (VII), though the procedure is obviously more complicated than the application of the formula (VI).

Arrangement of Work for Grouped Data

When the data are grouped, the value of k may be computed by the following formula:

$$k = \frac{N\Sigma(f\log x.\log y) - \Sigma(f\log x)\ \Sigma(f\log y)}{N\Sigma(f\log^2 x) - \Sigma(f\log x)^2} \qquad \text{(IX)}$$

To find $\Sigma(f\log x.\log y)$ *and* $\Sigma(f\log y)$*:* In order to find the value of $\Sigma(f\log x.\log y)$, lock the extreme left unit of the keyboard, depress the first value of log x and multiply it by the value of $f\log y$ for the same group or class. Repeat the same operation for all classes. The two numbers on the lower dial will be: to the right $\Sigma(f\log x.\log y)$ and to the left $\Sigma(f\log y)$. Clear the machine, leaving the extreme left unit locked.

To find $\Sigma(f\log^2 x)$ *and* $\Sigma(f\log x)$*:* To find the values in the denominator, $\Sigma(f\log^2 x)$ and $\Sigma(f\log x)$, which is also used in the numerator, depress on the keyboard the values of log x for the given class and multiply it by the value of $\Sigma(f\log x)$ on the same class. Repeat this operation for all classes. The values on the lower dials will correspond to $\Sigma(f\log x)$ to the left and $\Sigma(f\log^2 x)$ to the right.

These values allow the solution of the equation (IX).

Summary

A technique for machine computation of the relative growth constants b and k, in the equation $y = bx^k$, by the method of least squares is described.

The procedure of treating the ungrouped and the grouped data is given.

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CHANGES IN THE FREQUENCIES OF THE VARIATIONS OF *CEPAEA NEMORALIS* (LINNÉ)

THE literature which lists the variations of pentataeniate helices, particularly *Cepaea nemoralis* (Linné) and *C. hortensis* (Müller), is extensive, and breeding experiments have been conducted by Lang (1904), by Stelfox (1918) and by others. A systematic record of frequency changes in the variations produced by natural selection is apparently lacking, however.

The opportunity for such a study is excellent in the colony of *Cepaea nemoralis* at Lexington, Virginia. Howe (1898) recorded the frequencies of the patterns fifteen years after the introduction of the species from Europe. The present work is concerned with a comparison of frequencies determined in 1898 and in 1930 on a similar area. A comparison is also made between three areas in the same colony.

Although attention has been called to systematic methods of recording color variations, color seems of lesser importance than banding variations. Indeed, Trueman (1919) adequately states: "... they may be generally referred to two groups, namely, of red and yellow shells, which are discontinuous." Closer discrimination might justify a third group described by Reichert (1928) as absence of positive color factor. In the present consideration only banding variations are compared.

The collections for the present work were taken from three areas within the limits of Lexington, but separated by somewhat more than a quarter of a mile. The number of specimens in each of the three series (*X*, *Y* and *Z*) and the location of the area from which each series was taken are indicated in Fig. 1. The areas from which Howe's specimens were taken are indicated also. Howe's series *A* and *B* were taken a year apart in the same area. Series *C* was taken from the place of introduction.

The snails are abundant in all these areas, as, indeed, they are within the limits of the entire town south of Woods Creek. They have appeared in East Lexington in the past several years and are increasing rapidly, according to reports. The areal extent of the snails is probably more than twice what it was in 1898.

The system of formulation used to describe the patterns is a modification of that of Von Martens. The area of the whorl is considered as divisible into five zones, which increase in width downward from the suture and have their boundaries parallel to

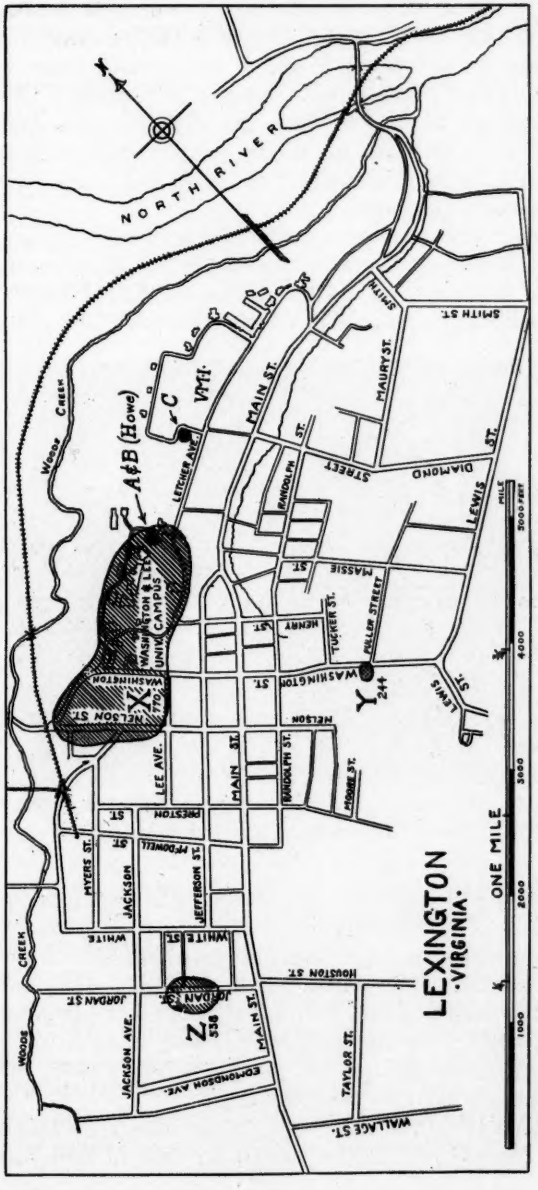

FIG. 1. Map showing location and relative size of areas from which the specimens were taken. The number of specimens in each series is indicated. The area of Howe's series *C* is the place of introduction.

the suture. All bands are referred to one or more of these zones (positions) and thus x's, which have been used to denote bands which could not be referred to any of the "usual" bands, have been eliminated. Although such a system of notation might not prove adaptable to cases in which the variability becomes extremely complex or where the occurrences of bands along zonal boundaries are not the exception, it seems adapted to comparisons of frequencies in this colony.

Ordinarily the bands vary in width both with reference to position on the whorl and on different specimens in a similar position. Centers of bands in positions 1 and 2 are ordinarily closer than in positions 2 and 3, etc. (Fig. 2), and this must be considered

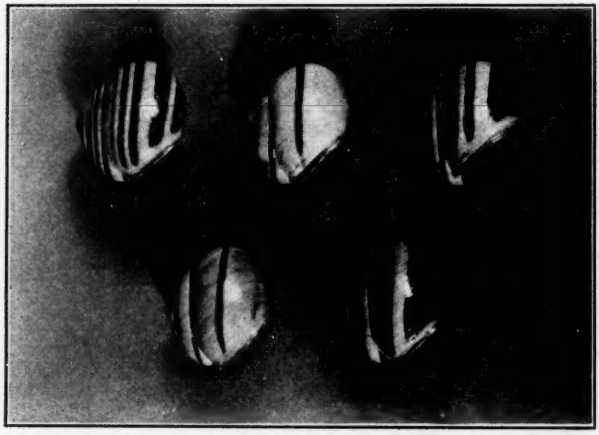

FIG. 2. Illustration of several varieties of patterns:
12345 00300 [12]3(45)
0030_3 [1(23)](45)

in determining the position. The absence of a band is indicated by 0 in place of the corresponding numeral. Other symbols used here are the following: (45) distinct fusion of bands in the fourth and fifth positions; [45] partial fusion of bands; $_3$ rudimentary band in the third position; 33 two bands in the third position; [22] two bands in the same zone but partially fused or less completely split, as the case may be; [3(45)] complete fusion of two bands which are partially fused to a third.

Table 1 shows pattern 00300 as seventeen times more abundant near the place of introduction (series X) than in more distant

TABLE 1
FREQUENCIES OF PATTERNS GREATER THAN ONE HALF OF ONE PER CENT.
(IN PER CENT.)

	Series *X*	Series *Y*	Series *Z*	*X*+*Y*+*Z*
00000	19.7	47.9	39.7	31.1
12345	17.5	16.0	24.0	19.5
00300	21.1	1.2	1.5	11.2
123(45)	7.4	9.8	8.5	8.2
123[45]	6.2	2.9	7.1	6.0
,2345	5.5	1.6	2.4	3.8
[12]3(45)	3.1	4.1	1.9	2.8
,,345	1.6	2.0	1.9	1.7
,23(45)	2.5	2.0	0.2	1.6
[12]3[45]	1.6	1.2	1.5	1.5
(12)3(45)	0.9	1.2	2.0	1.4
12045	1.6	0.0	1.3	1.2
,23[45]	1.4	1.2	0.4	1.0
10345	1.3	0.0	0.9	1.0
1,345	0.9	0.8	0.7	0.8
[12345]	0.5	0.8	0.6	0.6

locations, while 00000 has a frequency of approximately half that found in more remote areas. Pattern 12345 remains approximately the same in each series. Increases in frequency among the snails which have migrated some distance from the place of introduction appear in patterns: (12)3(45), ,,345, and 123(45) besides 00000. Decreases in frequency accompanying the migration appear in patterns: ,2345, [12]3[45], 12045, ,23[45], and 10345 besides 00300. Dominant potentialities seem most prevalent among the individuals which have migrated, since bandlessness has been demonstrated as dominant by Lang (1904) and by Stelfox (1918).

The frequencies of the bandless pattern in series *Y* and *Z* are considerably larger than for most of the European localities for which data are available for comparison.

Frequency changes brought about during thirty-two years are to be found by comparison of series *X* with Howe's series *A* and *B* (Table 2). Although the areas are not identical, the area of *X* represents a radial continuation in the same direction from the place of introduction and is inclusive of the area of *A* and *B*.

The bandless pattern has increased approximately five-fold. This is similar to the change which accompanied migration.

TABLE 2

COMPARISON OF PATTERN FREQUENCIES IN 1898 AND IN 1930 (IN PER CENT.)

	1898 (Howe)			1930		
	A	*B*	*C*	*X*	*Y*	*Z*
12345	40.0	41.1	31.6	17.5	16.0	24.0
(12)345	0.7	0.6	0.7	0.0	0.0	0.0
[12]345	*			0.1	0.4	0.0
123(45)	12.3	10.5	17.2	7.4	9.8	8.5
123[45]	*			6.2	2.9	7.1
(12)3(45)	4.0	4.4	5.2	0.9	1.2	2.0
[12]3(45)	*			3.1	4.1	1.9
[12]3[45]	*			1.6	1.2	1.5
(123)(45)	0.4	0.6	1.9	0.3	0.4	0.4
[123](45)	*			0.5	0.4	0.4
1(2345)	0.0	0.0	0.5	0.0	0.0	0.0
(12345)	0.4	0.1	0.8	0.0	0.0	0.0
[12345]	*			0.5	0.8	0.6
,2345	1.2	0.9	3.1	5.5	1.6	2.4
,23(45)	0.3	0.6	1.5	2.5	2.0	0.2
,23[45]	*			1.4	1.2	0.4
10345	6.0	6.2	2.5	1.3	0.0	0.9
,0345	1.1	1.1	2.2	0.1	0.4	0.0
1_2345	3.6	4.0	3.7	0.9	0.8	0.7
12045	1.3	2.0	1.2	1.6	0.0	1.3
120(45)	0.4	0.5	0.3	0.1	0.4	0.0
12_x45	0.9	0.6	0.4	0.3	0.8	0.6
$_{12}345$	0.6	0.5	2.9	1.6	2.0	1.9
00300	8.2	8.8	0.2	21.1	1.2	1.5
0030_x	0.9	1.0	0.1	0.1	0.0	0.0
00000	4.0	2.6	5.0	19.7	47.9	39.7
123_x45	1.1	1.3	0.9	†		
12_x345	0.6	0.4	0.2	†		
12[33]45				0.0	0.0	0.4
$(12)3_x(45)$	0.3	0.2	0.5	†		
[12]33(45)				0.0	0.4	0.0

* Howe did not distinguish between complete and partial fusion and consequently these patterns are included in the pattern immediately preceding.

† The system of formulation used here does not require the use of *x*.

Pattern 00300 has increased between two- and three-fold and this is the reverse of the condition among the creatures that migrated. Pattern 12345 is slightly less than one half as frequent as it was in 1898.

At the place of introduction Howe found the frequency of pattern 00300 to be 0.2 per cent., while the frequencies were approximately sixteen times as great only a short distance away. In 1930 the frequency of this pattern near the place of introduction had increased enormously, while in more removed areas the frequency had increased only slightly, comparatively. In comparing series

A with *B* with *C* and *X* with *Y* with *Z* it is noticeable that when the frequency of this pattern is large the frequency of the bandless pattern is proportionally smaller, and conversely. This suggests that pattern 00300 may represent a variety of secondary dominance, if dominance can be assumed to be increasing in all instances.

It is interesting that the three least intricate patterns (00000, 00300, and 12345) greatly predominate in frequency of occurrence. These patterns constitute 58.3 per cent. of series *X*, 65.1 per cent. of series *Y* and 65.2 per cent. of series *Z*. The frequency of these three patterns, taken collectively, in Howe's series are: *A*—52.2 per cent., *B*—52.5 per cent. and *C*—36.8 per cent. The sum of these three patterns is least near the place of introduction in both cases.

Comparisons of series *Y* and *Z* with *C* are indicative of changes produced by the combination of both factors, time and migration. Here the greatest increases in the bandless pattern appear.

Summary and Acknowledgments

Dominant characteristics have increased in the colony as a whole but at a less rapid rate near the place of introduction. There has been a marked decrease in the frequency of the more complicated patterns, and this is regarded as a decrease in the tendency toward development of variations through adaptation of the species to its new American environment. The bandless pattern has appreciably increased in frequency during the thirty-two-year period. There have been other changes of lesser consequence.

The writer is indebted to Professor W. D. Hoyt, Washington and Lee University, for constructive criticism of the work, and to Dr. W. Adam, Musée Royal d'Histoire Naturelle de Belgique, for reading the manuscript and for advice. The responsibility for the interpretations rests upon the writer alone.

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VARIATION OF THE DOUGLAS GROUND SQUIRREL IN DIFFERENT PARTS OF ITS RANGE[1]

INTRODUCTION

TAXONOMIC work by American mammalogists, *e.g.*, Anthony, Bailey, Grinnell, Osgood and others, has demonstrated that most mammalian species can be divided into subspecies or geographic races. Sumner (1920) in particular has shown by his work on the white-footed or deer mouse, *Peromyscus,* that even within a subspecies, localities differing in physical features are inhabited by local races exhibiting small but none the less detectable differences from one another.

> Thus, the two extremes, in respect to ear length, are presented by the Berkeley and La Jolla collections, both of which are generally assigned to the subspecies "gambeli," while the subspecies "rubidus," as regards several characters, presents at least three well-marked gradations, as we pass southward from Humboldt to Sonoma County.

These subspecific modifications in *Peromyscus* do not seem to be merely random variations but appear to be related to the environment. To quote Sumner further:

> If we consider only the coastal stations from San Francisco Bay northward (Berkeley, Duncan Mills, Fort Bragg, Eureka) which probably present a graded series in respect to both temperature and atmospheric humidity, we find likewise a similar gradation in respect to the mean width of the tail stripe and the mean length of tail, foot and ear. The suggestion lies close at hand that we have to do with some more or less direct influence of the environment, which in the course of time has modified hereditary characters of the animals dwelling at these various points.

If these morphological variations in the mice are in some way related to the physical and climatic factors of their environment, might we not expect to find these factors related to variations in

[1] Acknowledgment is made to Dr. R. R. Huestis, of the University of Oregon, for helpful suggestions in this work.

animal forms other than mice? If variations occurred in other animals would they be of the same general nature and extent? It was with these questions in mind that a series of Douglas ground squirrels, *Otospermophilus douglasii*, were taken from a number of different localities. While this animal is a rodent like the *Peromyscus* there is considerable morphological difference and its activities are diurnal not nocturnal.

Range

The range of this animal is indicated to give some idea of the geographical territory and possible range of variation in climatic and physical factors. The distributional data were compiled from Grinnell (1918), correspondence with I. N. Gabrielson, of the Biological Survey, miscellaneous sources and my own observations. The southern boundary of the Douglas ground squirrel lies just north of San Francisco Bay and extends from the coast to the Sacramento Valley. The eastern boundary follows north along the Sacramento River to Chico, thence northeast to the Nevada line and up into Oregon near Lakeview. Twenty miles north and east of Lakeview would probably mark the limits of their range in that region, but they extend westward from there to the coast. These ground squirrels do not extend very far north of Klamath Falls on the east side of the Cascade Mountains, but have worked their way up through all Western Oregon as far as the Columbia River, up the bank of that river on the south side and down on the east side of the Cascades about to the level of Redmond, Oregon. There may be some crossing over the Cascades as they have been seen above the 4,000-foot level on the western slope; but since the range is considerably higher than 4,000 feet and is for the most part densely wooded it is believed the Cascades act as a barrier. They do extend much farther east than The Dalles, Oregon, but are spreading northward across the Columbia River into Washington at Lyle and White Salmon. Grinnell (1918) considers Tehama County as the area of their greatest abundance in California. In Oregon they are very numerous in Jackson and Josephine counties and are also abundant in Wasco County. Fig. 1 shows their distribution in 1930.

Collecting Stations and Climatic Conditions

It is evident that an animal distributed over a territory of this extent is subjected to a variety of physical and climatic condi-

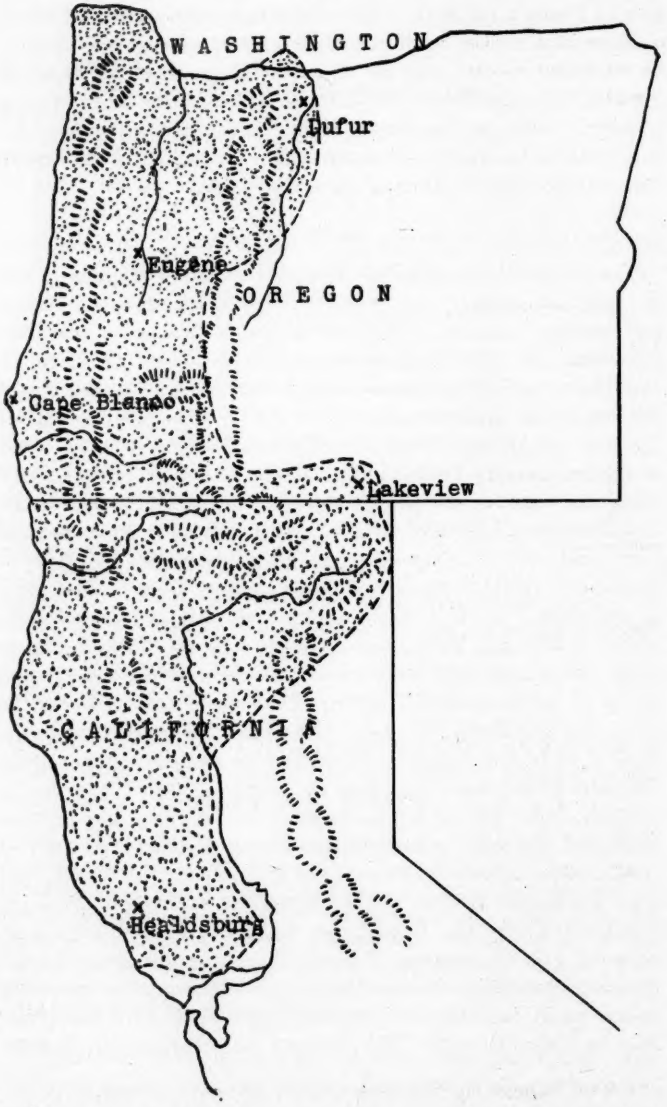

FIG. 1. Map showing distribution of *Otospermophilus douglasii* (shaded) in Oregon, California, and Washington. The localities from which series of specimens were taken are indicated by an "x."

tions. Three localities of climatic extremes were selected: (1) on the coast near Cape Blanco, Oregon, where it is humid with relatively narrow temperature ranges; (2) in eastern Oregon near Dufur, an arid region with wide temperature ranges and more clear or partly clear days; and (3) Eugene, Oregon, with intermediate climatic conditions. Series of specimens, at least thirty, were taken at each locality. The following table gives a summary of some of the physical and climatic conditions at the localities where the three large series of specimens were taken.

TABLE I

CLIMATIC AND PHYSICAL CONDITIONS AT COLLECTING STATIONS

	Cape Blanco	Eugene Oregon	Dufur Oregon
Elevation (ft.)	200	500	2000
Annual precipitation (in.)	80	39	15
Dry season precipitation	3.25	2.5	1.1
Dry season mean temp. (F.)	57.1	63.7	62.5
Annual mean temp.	52	51.6	46.4
Frostless season (days)	260	199	147
Clear days	167	125	179

The dry season is assumed to be the months of June, July and August and is included because the squirrels are quite active during these and the preceding two months. The climatological data presented above are taken from the U. S. Weather Bureau Summaries and represent the average of conditions for the last twenty or more years.

Smaller series of specimens were taken near Lakeview, Oregon, and Healdsburg, California, as a check on the species at the margins of its range. Fig. 1 shows the location of all collecting stations.

STRUCTURAL VARIATIONS

Sumner in his studies of variation in *Peromyscus* found the parts most likely to show variation were pelage color, tail length, width of tail stripe, foot length and depth of foot pigmentation. Pelage color, width of dorsal tail stripe and depth of foot pigmentation seemed correlated with one another geographically. Other variations mentioned by Sumner were ear length, number of tail vertebrae, pelvis, femur and skull lengths.

A large number of structural features of the ground squirrel in addition to those usually recorded were studied. Various skeletal, skull and internal structures measurements were made, tail vertebrae and palatine ridges counted, and pigmentation of parts noted. Most of these data showed no significant differences, and others not deemed reliable have been omitted. In all about two hundred specimens were measured and the skins preserved. The measurements of females only are tabulated here because the number of male specimens taken were less than one third the females and being relatively larger in size would break up the homogeneity of the female group. The measurements of the males, however, were all averaged and showed the same characteristics as those of the females from the same locality.

The following table shows the measurements and characteristics considered significant of the series obtained from the coast region, eastern Oregon and Eugene. Standard deviation and probable error are included.

TABLE II

MEAN MEASUREMENTS SHOWING VARIATION OF DOUGLAS GROUND SQUIRRELS FROM DIFFERENT REGIONS

	Coast No. 31 ♀	Eugene No. 30 ♀	Eastern Oregon No. 33 ♀	Different Coast and Eastern Oregon
Mean body length	241.05±1.46	243.3 ±1.56	257.5 ±1.23	16.0 ±1.90
Standard deviation	12.05±1.03	12.70±1.10	10.45±0.86	
Mean tail length	196.0 ± .81	191.1 ±1.18	183.5 ±1.23	12.5 ±1.47
Standard deviation	6.70± .57	9.57± .83	10.50± .87	
Mean ratio tail/body	81.23± .54	78.43± .55	71.37± .56	9.86± .77
Standard deviation	4.48± .38	4.46± .38	4.78± .39	
Mean foot	56.0 ± .182	57.4 ± .256	56.5 ± .147	.5 ± .299
Standard deviation	1.50± .128	2.21± .193	1.25± .104	
Mean ear	26.8 ± .155	27.6 ± .134	28.3 ± .102	1.5 ± .185
Standard deviation	1.28± .109	1.16± .101	.86± .072	
Relative pigmentation of pelage	Dark	Medium	Light	

Relative to pigmentation of pelage, dark is defined as a more general brownish color, and light as more grayish, especially

noticeable on the shoulder patches and under parts. Although the pelage is lighter the dark dorsal shoulder wedges appear to be slightly more accentuated in the eastern Oregon forms. That average color differences exist in a series of skins from different localities was shown by a random spreading out of skins from each locality in respective rows; then calling in separately four persons ranging from the janitor to an experienced field mammalogist, who were not informed as to the place of origin of the skins and asking them to point out the lightest and darkest of the three rows. The selection made by each individual was the same and confirmed my selection. Fig. 2 shows representatives from each locality.

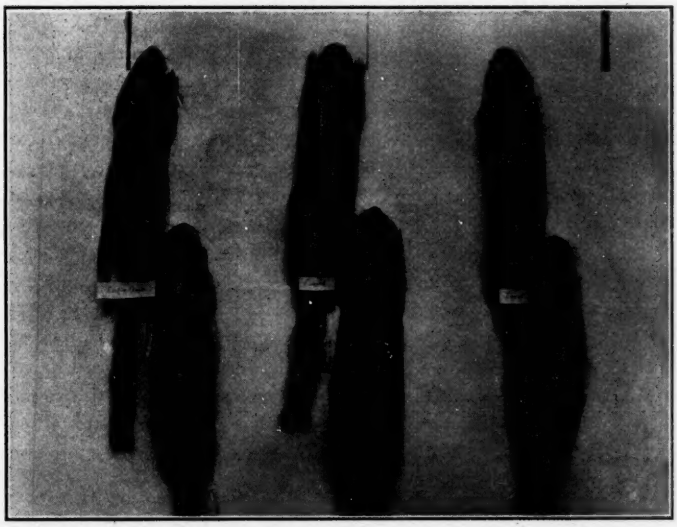

FIG. 2. Representative specimens taken from Eastern Oregon, Eugene and the coast. The shoulder patches appear lighter in the forms from Eastern Oregon, and the general pigmentation of the coast forms appears darker than the others.

A study of the table of variations shows that the eastern Oregon forms differ in several respects from the coast forms. They have less pigmentation in the pelage, the tail is considerably shorter, while the body is longer. The difference in the means of the tail/body ratio is approximately twelve times the probable

error of the difference. The ear length was greater by 1.5 mm. The eastern Oregon forms also appeared more alert and active than those on the coast. The coast forms show the converse of the character differences found in the eastern Oregon forms. The foot measurements showed but little difference in the series from different localities. The differences indicated by the above table are probably not of sufficient magnitude to be considered of subspecific value by the average mammalogist.

DISCUSSION

When the table of physical and climatic factors is checked against the table of variations, certain correlations are apparent. The forms from the semi-arid eastern Oregon, with its greater amount of sunshine, wide temperature range but higher temperature during the active season, shows greater body length, shorter tail and longer ears than do the forms from the more humid coast region, with its cooler weather and less sunshine, while Eugene, with intermediate climatic conditions, shows an intermediate gradation in structure. A study of the characteristics of a small series from Lakeview, Oregon, and Healdsburg, California, shows their resemblance to the Eugene forms, except in pelage pigmentation, which was nearer like the eastern Oregon forms. If physical and climatic factors are correlated with the characteristics considered these would be the expected results.

This gradation of structure in the ground squirrels with gradation of physical factors parallels in a general way the results of Sumner on *Peromyscus,* although the structures involved are not all the same ones; however, this would hardly be expected since two different families are involved, one a diurnal animal, the other nocturnal.

The character differences in the eastern Oregon forms must be of comparatively recent occurrence, since according to Grinnell (1918) the species is not known to have existed in historical times as far north as the Columbia River.

When one seeks the explanation of these variations he invades a realm of speculation and conflicting opinions. Most biologists probably account for variations in one of several ways: through adaptive modification of random variations or mutations, brought about by natural selection in the environment; or of less vogue, by inheritance of gradual modification brought about by direct effect of the environment; and finally an orthogenic tendency in

the germ-plasm to vary with the environment unimportant or at most only slightly directive.

One hesitates to ascribe any survival value to the variations shown in the table. Robson (1928) is of the opinion that structural differences must be of genetic, better familiar value before they are positive enough to be of selective value. In this case where a gradation in structure occurs with a gradation in physical factors of the environment the tendency is to attach considerable importance to environmental influence.

Growth appears to be the result of one or more factors or forces of development resulting in increase in size. It seems reasonable to suppose, according to Newton's third law, that the initiating influence is met by an equal and opposing force or forces. The initiating physio-chemical force might be thought of as of hormone nature or due to the effect of the more remote chromosomal determiners. The opposing force would be the inertia or resistance to the development of the structure under consideration and might serve as a mechanism for modifying the causative force. The balance between the opposing influences might be directly or indirectly susceptible to environmental influence, which, acting through many generations, might permanently shift the equilibrium point forward or backward with resulting decrease or increase in the size of the part considered. Different species would be expected to vary in their susceptibility to environmental stimulus, and variability would differ in different parts of the same animal.

Summary

1. Collections of Douglas ground squirrels were made in regions of different physical and climatic conditions in Oregon.

2. A study of the characteristics of these different groups of squirrels showed a significant difference in tail length, body length, ear length and pigmentation of pelage.

3. The variations of structure were correlated with variations in the climatic and physical factors and support in a general way the findings of Sumner on *Peromyscus*.

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THE CULTURING OF FRESH-WATER PROTOZOA AND OTHER SMALL INVERTEBRATES

THE need for more dependable supplies of living specimens in the general biology course at Washington Square College has led us to work out many modifications and new procedures in the methods of culture. Some of the results have been so favorable, as regards more rapid growth and longer life of the cultures, that the writer has been urged to submit these notes.

Amoeba:[1] The following method has given a large proportion of successful cultures which achieve a very dense maximum growth in from 3 to 4 weeks and do not require subculturing for from 8 to 10 weeks. It differs from previous methods chiefly in the use of agar[2] and the slight modification of the salt content of the culture solution.

Prepare finger bowls by covering the bottom with a thin (1–2 mm) sheet of agar. This is done by pouring a warm, filtered, aqueous 0.75 per cent. solution of powdered agar into each bowl. While the agar is still soft imbed five rice grains, evenly spaced. The finger bowls and pipettes are previously washed thoroughly in hot water, and the rice heated (10 minutes) in a dry test-tube kept in boiling water; these are precautionary measures against contamination.

[1] (a) H. W. Chalkley, *Science*, 71: 441, 1930, and (b) D. M. Pace, *Arch. Protistenkunde*, 79: 133, 1933, have previously reported two methods for Amoeba. Our medium differs little from Chalkley's, but the method in its entirety has given better results.

[2] The use of an agar layer on the bottom of the dishes was originally suggested by Dr. Robert Chambers for the purpose of anchoring the rice grains, about which the amoebae tend to congregate. Mr. M. Sheib, working for Dr. Chambers, has been very successful with this method. The writer came upon it independently and is of the opinion that the augmented growth is due to the increased surface available to the amoebae for securing their prey, although some component, added via the agar, may be involved.

About 50 amoebae, together with 10 cc of the medium in which previously they have been growing, are introduced into each bowl and 30 cc of the general culture solution (solution A)[3] are added. Thereafter, every three days, 20 cc of solution A are added to each bowl until the total volume is from 80 to 90 cc.

When maximum growth has been attained and a culture shows signs of waning, it may be replenished by adding 10 cc of solution A and one rice-grain (preheated).

In a day or two after starting a culture the agar layer becomes detached from the bottom of the vessel and the amoebae grow in layers on its upper and lower surfaces and also on the glass surface.

In about two months, it is advisable to subculture by dividing the contents of each bowl, exclusive of the rice-agar, into four parts, pouring each into a freshly prepared finger bowl and adding an equal quantity of solution A. From here the procedure is the same as before.

If the original source of amoebae is limited, as is the case when they are collected from the field,[4] it is necessary to modify the method slightly by starting the cultures in smaller dishes, *e.g.*, Syracuse dishes instead of finger bowls. This apparently gives a better initial concentration of the amoebae and makes the change of culture conditions less abrupt.

The Syracuse dishes are prepared with an agar film in which two rice grains are imbedded. Introduce the available amoebae with 4 cc of the water in which they were collected and 4 cc of solution A. In successful cases there will be a rapid proliferation and, when from 200 to 300 animals are present, the contents (minus the rice-agar) is added to a rice-agar bowl with 30 cc of culture solution A. The steps from here are the same as before.

The cultures were maintained at 19–22° by stacking the finger bowls in a sheet metal container, placed in a sink in which tap water was kept circulating to the level of the highest finger bowl. Even better growth has been obtained at 17–19° C., but it was easier to maintain the former temperature.

[3] General culture solution (Solution A)—NaCl 1.20 gms, KCl 0.03 gms, $CaCl_2$ 0.04 gms, $NaHCO_3$ 0.02 gms, Phosphate buffer solution having a pH 6.9–7.0, 50 cc, distilled water to 1,000 cc. For use dilute this 1: 10. This solution maintains a fairly constant pH of about 7.0 and serves well not only for Amoeba, but also for general use.

[4] In ponds, from beds of Vaucheria, Hydrodictyon, from the undersides of Castalia, Lemna and Spirodela leaves.

Arcella: The foregoing technique has also been very successful for Arcella. In this case, however, temperature control is not necessary.

It is detrimental to have Stentor, Paramoecium, large hypotrichs, Philodina or Stenostomum in cultures of Amoeba or Arcella. A culture in which these organisms have gained ascendancy should be discarded, and precautions should be taken against similar contamination of other cultures. Chilomonas and Colpidium, while not detrimental in moderate populations, should not be allowed to proliferate to the point where a culture becomes cloudy with them. Mold usually grows on and about the rice and does not seem detrimental, although at times it is annoying since it is hard to disentangle the amoebae from the mycelium.

Actinosphaerium: For this organism best success has been obtained by insuring the presence of Paramoecium, Stenostomum or Philodina, which apparently serve as prey. To 30 cc of solution A in a rice-agar bowl add 30 cc of a dense culture (see below) of any of these organisms and inoculate with from 5 to 10 Actinosphaeriae. Maintain in diffuse light and replenish with more of the food organisms as required. Prolific cultures have usually been obtained in about two weeks.

Stylonichia and Oxytricha: For these hypotrichs and certain others the presence of Chilomonas seems advantageous. Before introducing the desired ciliate, allow about 30 cc of Solution A in a rice-agar bowl, inoculated with 10 cc of a Chilomonas culture (see below) to stand from 3 to 5 days. Swarming cultures have usually been obtained within 2 weeks.

Chilomonas: Another technique, applicable to this organism as well as several other protozoa, is the following. A thin smooth paste is prepared by grinding .5 gm of the boiled yolk of a fresh hen's egg with a small amount of distilled water. This is added to 500 cc of distilled water and the mixture, after standing two days, is inoculated with the original Chilomonas culture. If such a culture is not available, spontaneous inoculation will occur if the culture jar is left uncovered, since cysts of Chilomonas seem to be omnipresent.

Paramoecium, Colpidium, Colpoda, Euplotes: These ciliates have done well on the egg yolk medium when Chilomonas is provided as prey. Start a Chilomonas culture as previously directed and inoculate with 10 cc of a culture of the desired ciliate, three times, on the 4th, 6th, 8th day after starting. Dense maximum

growth has usually been obtained in 2 weeks and subculturing has been necessary about every month.

Didinium: The organism will thrive exceedingly well when introduced into one of the Paramoecium cultures described above. As the Paramoecium diminishes in one culture a fresh one should be available for inoculation with the Didinium. In fact it is advisable to keep Paramoecium cultures in a separate room; otherwise it is difficult to avoid contamination with Didinium.

Euglena: To 100 cc of a modified Kleb's solution (Solution B)[5] in a white glass battery jar add 40 rice grains (boiled from 5 to 10 minutes) and 900 cc of distilled water. The foregoing medium is allowed to stand for about five days. The jar is then placed in indirect sunlight (the direct rays of the sun should not strike this culture for more than an hour a day), and inoculated with Euglenae three times (10 cc of a dense Euglena culture) at three-day intervals. If an old Euglena culture is available the organisms may be found encysted on the sides of the vessel, and it is of great advantage to inoculate the cysts along with the free Euglenae. Starting ten days after the initial inoculation, growth may be accelerated by adding (3 times, at weekly intervals), 25 cc of Solution B and 10 mg of the tryptophane powder. The further addition of 5 grains of boiled rice each month will serve thereafter to maintain the culture. Large ciliates and rotifers are detrimental.

Hydra: These animals are maintained in great numbers in balanced aquaria when they are fed constantly with any of the Entomostraca mentioned below.

Platyhelminthes: Stenostomum may be cultured using the method used for Paramoecium.

Planaria are cultured in enameled pans containing clear pond or spring water; they are fed with boiled egg yolk or fresh liver, care being taken to remove the excess food at the end of a few hours before putrefaction occurs. The Planaria are cut transversely when they reach the size of about 8–12 mm with a No. 00 cover glass; regeneration occurs rapidly.[6]

Rotifera: Philodina is easily cultured by either the method used for Stylonichia or for Paramoecium.

[5] Modified Klebs' Solution (Solution B): KNO_3 .25 gm, $MgSO_4$ .25 gm, KH_2PO_4 .25 gm, $Ca(NO_3)_2$ 1 gm, Bacto-Tryptophane Broth (powder) .010 gm (Digestive Ferments Co.), distilled water to make 1,000 cc.

[6] This method for Planaria does not differ significantly from the procedure recommended by the commercial houses which supply this organism.

Entomostraca: Cyclops, Cypris and Daphnia have been cultured with moderate success by the following method: Two grams of egg yolk, ground into a paste, are added to a gallon jar filled with green pond or aquarium water. This is allowed to stand for about three days and then inoculated with small protozoa (any species not larger than Colpoda). Finally, the organism to be cultured is introduced—for Cyclops, a few males and egg-bearing females will suffice; for Daphnia and Cypris, as many individuals as possible are added. Within a month successful cultures will show organisms in abundance, a condition which will last another two weeks.[7]

Annelida: Microdrilli, such as Nais, Aelosoma and Dero, have responded splendidly to culture in 30 cc of Solution A (footnote 2) added to rice-agar as used for Amoeba. The medium in this case, however, should stand for three days before inoculating with the annelids (about 5 will suffice). The number of these organisms can be increased by using larger vessels, *e.g.*, allowing for more fluid and increasing the surface area of the agar.

To obtain initial supplies of a number of the foregoing organisms, the following methods are suggested. (a) Water weeds, brought from the field, are packed into a battery jar and covered with some of the pond water in which they were found. The jars are then placed in diffuse light and when decay begins many of the organisms will be found gathering at the surface of the water. (b) Green "surface blooms" in ponds may provide good sources of Euglenidae. (c) Pond water placed in battery jars with from 20 to 40 grains of boiled rice, and allowed to stand, will show a succession of forms and the desired ones can be isolated in Syracuse dishes in which the conditions are made similar to those of the mass cultures. Such small cultures, then, of course, provide the sources from which to start the large ones.

In general, the room which is being used for culturing purposes should be kept free of toxic vapors such as xylol, formalin and alcohol. Phenolic sterilizing preparations, *e.g.*, commercial "CN," must be avoided. The temperature in such a room should never exceed about 22° C.

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[7] For Daphnia, the cultures require a temperature of from 17 to 19° C. This was obtained by circulating cold water through a coil of glass tubing (6 to 8 mm), set within the gallon jar.

CHROMOSOME NUMBERS IN RELATIVES OF *ZEA MAYS* L.

DURING the last five years the writers have collected and made cytological studies of a number of relatives of *Zea mays*. The work is still in progress but several very interesting observations on the numbers of chromosomes, which seem worth reporting at this time, have been made.

The numbers found to date are given in Table 1:

TABLE 1

	Gametic numbers	Somatic numbers
Euchlaena mexicana Schrad.	10	20
Euchlaena perennis Hitch.	20	40
Tripsacum dactyloides L.		
Collected at Angleton, Texas	18	36
Collected at Manhattan, Kansas	18	36
Collected at Atchison, Kansas	18	—
*Collected at Nacogdoches, Texas	36	72
*Collected at Alpine, Texas	36	—
*Collected at New Haven, Conn.	36	72
*Collected at Miami, Florida	36	72
**Tripsacum laxum* Nash	36	72
**Tripsacum latifolium* Hitch.	36	72
**Tripsacum pilosum* Scrib. and Merr.	36	72
Coix lachryma stenocarpa Oliver	—	20
Coix lachryma—jobi L.	—	20
Sclerachne punctata Brown	—	20
Polytoca barbata Stapf	—	20
Manisuris cylindrica (Michx.) Kuntze	9	18

* These forms are regarded as autotetraploids. Some plurivalent chromosomes are formed by them during meiosis, and slight variations in their chromosome numbers were found.

The chromosome numbers of several of these forms have been reported previously by other investigators. Kuwada (1919) reported *Coix agrestis* Lowr. as having the unreduced number 20. He also found "Euchlaena aus Süd-Florida" to have 10 as its reduced number and 20 as its unreduced number. Longley (1924) found the reduced numbers in certain relatives of *Zea mays* to be as follows: *Euchlaena mexicana* Schrad. 10, *E. perennis* Hitch. 20, *Coix lachryma—jobi* L. 10, *Tripsacum laxum* Nash

35, *T. dactyloides* L. 35, and *T. pilosum* Scrib. and Merr. 35. The numbers reported in the present paper apparently are in agreement with those reported by other investigators, with the exception of those reported by Longley on the species of Tripsacum.

The number 10 is possibly the most frequent gametic number in the tribe Maydeae (Tripsaceae) and some writers have considered it to be the basic number. When this is done, however, the number 5 in *Coix aquatica* requires an explanation. Many members of the tribe Andropogoneae also have the reduced number 10. Tripsacum and Manisuris seem to be exceptions in their respective tribes in having 9, or multiples of this number, as their reduced number of chromosomes.

Manisuris cylindrica (Michx.) Kuntze, also known at present as *Coelorachis cylindrica* (Michx.) Nash, *Rotboellia cylindrica* Torr., and *Rotboellia campestris* Nutt., has been regarded generally as belonging to the tribe Andropogoneae because it has many morphological characteristics of that tribe. But it also has some characteristics of Tripsacum, and the finding of 9 to be its gametic chromosome number again suggests its relationship to Tripsacum. A history of the nomenclature of this species shows that in addition to its present names it was regarded by early botanists as *Tripsacum cylindricum* Michx. and therefore as a member of the tribe Maydeae. It differs strikingly from Tripsacum in that it has perfect flowers. Such differences, however, can be found among close relatives in other families, such as the species and varieties of Fragaria. Here are often found, even within the same genus, forms having perfect or imperfect flowers. The presence of perfect flowers in Manisuris and the absence of them in Tripsacum does not seem, therefore, to be justification for considering their relationship to be very remote.

In the tribe Maydeae, Tripsacum usually has been regarded as occupying an isolated position, but recently it has been hybridized with both Euchlaena and Zea (Mangelsdorf and Reeves (1931)). If Manisuris is correctly assigned to the tribe Andropogoneae, as it is generally regarded to be, we should consider the tribes Maydeae and Andropogoneae as being related through Tripsacum and Manisuris.

Avdulow (1931) reported *Rotboellia glandulosa*, a close relative of *Manisuris cylindrica*, as having 54 (a multiple of 9) chromosomes in its somatic cells. He suggested, however, that *Tripsacum dactyloides* may have 80 somatic chromosomes rather

than 70 or 72, and that its basic number is therefore probably 10. Also, he did not consider *Tripsacum dactyloides* as being closely related to the other Maydeae, not being aware that it had been hybridized with Zea and Euchlaena.

A full account of the present investigations will be published in the future.

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THE ASSOCIATION BETWEEN COLOR AND SIZE IN MICE

SOME four years ago the writer (1931a) reported in this journal an association in inheritance between larger size in certain quantitative characters and brown coat color in the back-cross generation of a mouse species cross involving a highly inbred strain of large *Mus musculus*, with the recessive genes for dilution, brown and non-agouti (dba), and small *M. bactrianus* with the corresponding dominant alleles for intensity, black and agouti (DBA^w). The tendency for the larger size and brown coat color derived from the *musculus* parent to remain together in the back-cross hybrids was interpreted as genetic linkage between the gene for brown and genes influencing growth located on the same chromosome, the first example of linkage between qualitative and quantitative characters demonstrated in mammals. Subsequently (1933) such linkage was confirmed by F_2 data, while more recently (1935) mean weights of additional back-crosses—in all of which the same two parental strains were used—again supported the previous conclusions.

In a recent number of this journal Feldman (1935) has published some interesting data, which likewise illustrate a tendency for brown pigmentation and greater weight to remain together. He interprets his findings, however, as due to a physiological effect of the brown gene itself and not to linked size genes, incidentally favoring the same explanation for the writer's observations described above.

In Feldman's experiments comparisons were made between black and brown segregates in three different stocks: (1) A small race derived from the introduction of brown into a wild-caught, presumably smaller, strain of house mice; (2) a large race of black and tans (a^t) carrying both black and brown; (3) the first generation hybrids of the two preceding. In all three stocks he found browns slightly heavier than blacks, the difference averaging 3.9 per cent. in males and 3.1 per cent. in females.

Although the difference between black and brown mean adult weights is so slight in each contrasted pair of values as to be markedly below the level of significance, nevertheless the deviations in all six pairs at all ages are in favor of the brown animals, thus manifesting an unusual consistency scarcely explicable by chance alone, such a possibility being practically precluded by the data given.

Unfortunately, Feldman is not explicit in describing the procedure followed in obtaining the black and brown animals compared, merely stating that both colors are present in the stocks. Since in all three experiments the Bb and bb mice weighed are present in approximately equal numbers it seems probable—unless the blacks used constitute a selected sample from a larger population—that recessives were mated to heterozygotes. If such was the case, it is obvious that linked genes might conceivably account for the results in stock 3 at least.

But regardless of the true explanation of Feldman's results, granting that the differences are real and not fortuitous, it appears improbable that the writer's data can be best interpreted by assuming a physiological effect of the gene *b*.

It will be recalled that all the investigations which led to the adoption of a linked gene explanation for the larger size associated with brown coat color in back-cross mice involved the same two parental strains, large dba *musculus* and small DBA^w

bactrianus. If the larger size be due, not to genes influencing growth linked with b, but to a favorable physiological effect of the b gene itself, brown segregates should be heavier than their black sibs, regardless of the brown stock employed. This should hold true, whether the genes for brown are identical or whether one is a genic isomer of the other. Since the latter alternative, possible on purely theoretical grounds, would not be amenable to proof in mice it seems safe to assume that all genes for brown at the same locus and having the same phenotypic effect on coat color are identical. It now appears, however, that brown is not necessarily always associated with larger size in inheritance.

An unrelated inbred strain of large leaden, brown, non-agouti ($d_2$ba) *musculus* was mated to the same strain of *bactrianus* used in the earlier investigations. Male F_1's were then back-crossed to females of the $d_2$ba strain, producing heterozygous blacks and homozygous browns in approximately equal numbers. Table 1 presents the mean weights with their standard errors at 181 days. The females included are all nulliparous.

TABLE 1

Sex	No.	Type	Mean weight in grams	Difference
♂♂	55	Bb	29.69 ± .33	.33 ± .53
	51	bb	30.02 ± .41	
♀♀	38	Bb	23.61 ± .33	.45 ± .46
	44	bb	24.06 ± .32	

The differences present are obviously of no importance, since they are less than their standard errors in both pairs. This lack of association between size and color in the back-cross hybrids when the $d_2$ba strain was used is at variance with the significant differences between blacks and browns when the b chromosome came from the dba strain of *musculus*. The results of all experiments, including the earlier indecisive ones (1931) conducted under unfavorable conditions, comprising a total of 797 back-cross mice, have shown that the average bb male weighed 6.1 per cent. more at 181 days than the average Bb, with the corresponding figure for the females 6.7 per cent. These amounts,

when the dba strain was involved, are in marked contrast with the insignificant excesses manifested by browns in the table above. Such divergent results are more easily explained by the assumption that the genes linked with b which influence growth have become fixed in the dba strain but are absent in the $d_2$ba than by assuming that the gene for brown in the latter mice, while retaining its effect on pigmentation, has in some manner lost its hypothesized beneficial physiological influence on growth.

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